The Journal of Laboratory and Clinical Medicine

VOL. XVI ST. LOUIS, MO., JULY, 1931 No. 10

CLINICAL AND EXPERIMENTAL

CHEMISTRY AND APPLICATION OF CAMPIODOL (IODIZED RAPESEED OIL) IN ROENTGENOGRAPHY*

BY GEORGE W. RAIZISS, PH.D., MARK A. GLASER, M.D., AND LE ROY W. CLEMENCE, B.S., PHILADELPHIA, PA.

IN THE roentgenographic exploration of body cavities, substances must be employed for visualization which, in addition to satisfactory shadow-casting properties, fulfill the requirement of low toxicity. Unfortunately, solutions of electrolytes do not satisfy this requirement, and are too toxic to be of use clinically. The consequent search for suitable inert substances has culminated in the work of Sicard and Forestier[1, 2] who proposed the use of iodized oils in 1923. Such oils have since received wide application for purposes of photographic visualization.

The experimental study presented in this paper was therefore centered on oils. The ability of certain oils to absorb iodine depends on the unsaturated property of some of the carbon atoms composing the glyceryl esters present in the oils. These unsaturated glyceryl fatty esters vary in their degrees of iodine absorption, and those with the highest amount of unsaturation will naturally absorb the largest amount of iodine. The iodine will be held in firm union once it has combined with the free valence linkage of the carbon atom.

The purity and stability of the iodized oils depend on the method of their preparation. Various methods of introducing iodine were tried by us: (1) the mixing of elemental iodine and the raw oil by rapid stirring and agitating. An excess of iodine was used and the mixture finally filtered. The iodine was not well absorbed and the final product rapidly decomposed. (2) The introduction of gaseous hydriodic acid produced by passing HCl vapors over CaI_2,

*Received for publication, January 5, 1931.

Read before the Division of Medicinal Chemistry of the American Chemical Society, at Cincinnati, September, 1930.

directly into the oil (D.R.P. 135,835), until a definite gain in weight is obtained. This had its disadvantages, inasmuch as the largest amount of iodine could not be absorbed, because the double bond was saturated both by hydrogen and iodine. Thus:

$$HI + (-C = C-) \rightarrow -\underset{H}{C}-\underset{I}{C}-$$

As a result only one-half of the total absorptive power was taken up by the iodine. It was also an expensive and unhandy method, the apparatus for the generation of hydriodic acid gas being quite complicated in large scale production. (3) The adaptation of analytical methods used for the determination of iodine numbers of oils, such as the Hanüs[3] or Hübl[4] methods, resulted in the production of oils with the maximum amount of iodine. The double bonds are saturated, as in both cases an excess of iodine is used. Our problem was to isolate the iodized product and to eliminate any excess of iodine. We finally adopted a variation of the Hübl method, using mercuric chloride as a catalyst. The solvent for the oil was chloroform, the complete removal of which from the final product is not very easy. As a matter of fact, traces of chloroform have a tendency to adhere to the iodized oil, rendering it liable to decomposition and a consequent increase in toxicity. The employment of ether instead of chloroform is of advantage.

The following is the description of the procedure which has thus far given us the least toxic product and has been the most satisfactory in respect to iodine content, color, and consistency.

A solution of 100 grams of iodine in 1000 c.c. of ethyl alcohol is mixed with a solution of 120 grams of mercuric chloride in 1000 c.c. of ethyl alcohol and the whole is added in a very slow stream to 100 grams of rapeseed oil dissolved in 600 c.c. of chloroform. The resulting reaction mixture, which contains an excess of iodine, is permitted to stand for about six hours and is then vigorously mixed with 5 gallons of distilled water for about five minutes. At the end of this time the chloroform solution of the final product separates from the aqueous solution containing the extra iodine and other ingredients. The latter is siphoned off, and the remaining chloroform solution is first freed of precipitated mercuric iodide by filtration, and then washed with 1 per cent potassium iodide solution until it becomes almost colorless, thereby indicating that the last traces of free iodine have been removed.

The chloroform solution is then washed with water until free of potassium iodide, and finally rendered free of all traces of adherent water by the addition of anhydrous sodium sulphate. After filtering off the latter the chloroform solution is further purified by the introduction of "nuchar," filtered again, and most of the solvent removed from the filtrate by distillation in vacuo. From the residue the last traces of chloroform are eliminated by slight warming in the water-bath at ordinary pressure.

Ether can be substituted for chloroform, using one and a half times its volume. We were able to reduce the quantity of iodine in this method so

that only a slight excess was necessary. This was of considerable advantage, as it entailed the use of much smaller quantities of potassium iodide in removing the slight excess of halogen. Furthermore, ether is more readily removed by distillation, since its boiling point is lower than that of chloroform.

The selection of the oils used depends upon a low specific gravity, high iodine value, and low viscosity. The fish oils were tried first. Cod liver oil was deemed particularly adaptable because of its well-known ease of absorption in the human body. After iodization, however, the oil became more viscous, heavier, and darker in color, being a deep amber, but still remained clear. The iodine content was 55 to 56 per cent. After a short time it gradually turned dark, finally becoming a thick black opaque mass. Menhaden oil was very similar to cod liver oil, giving an iodized product of 51 to 52 per cent iodine content. This also decomposed on standing. Among the vegetable oils tried were sweet almond oil, 40 per cent I; peach-kernel oil, 35 per cent I; maize oil, 40 per cent I; rapeseed oil, 43 to 45 per cent I.

Rapeseed oil (Colza oil) is derived from the seeds of Brassica napus, Brassica campestris, Brassica rapa. It is a yellow, almost odorless oil with a specific gravity of 0.914 and an iodine value of 93.5 to 105.6. It is composed of the glycerides of stearic, erucic, and an oleic acid. Iodized rapeseed oil, to which we have given the name campiodol, is a light yellowish, semiviscous, transparent oil with a slight aromatic odor and oleaginous taste. It contains 43 to 45 per cent iodine and its specific gravity is 1.2 to 1.3 at 20° C. It is soluble in chloroform, ether, benzene, and carbon bisulphide and insoluble in water and alcohol. This chemical compound deteriorates very slowly upon exposure to light and heat, some samples remaining stable for over a year, and upon radiographic exposure an intense shadow is obtained, so much so that dilution products may readily be made without destroying its clinical value.

Dilution products can be prepared either by the use of rapeseed oil, olive oil, or ethyl esters of olive oil. The latter was prepared as follows: 1000 gm. of purified olive oil, 2500 c.c. of absolute ethyl alcohol and 30 gm. of sulphuric acid are mixed and refluxed on a steam bath for eight hours. This is then poured on 5 gallons of distilled water and mixed. The oil layer is separated and washed several times with distilled water. It is dried with sodium sulphate, purified with "nuchar," filtered, and distilled off at 195-205° C., 2 mm.

Campiodol emulsion is prepared by mixing thoroughly 100 c.c. of iodized rapeseed oil with 100 c.c. of 35 per cent purified acacia solution and 200 c.c. of distilled water. The best procedure is first to mix the acacia solution with the campiodol in a glass vessel until a thick white homogeneous mixture is obtained; the distilled water is then added in a very slow stream and agitated rapidly for about fifteen minutes.

From Table I it is interesting to note that the ethyl esters of olive oil have a very low specific viscosity. Campiodol emulsion is next, also being of low viscosity. Campiodol 4:1 has a specific viscosity of 20.3 which is lower than that of other iodized oils generally used in roentgenography.

TABLE I

VISCOSITY COMPARISONS TAKEN WITH STORMER VISCOSIMETER AT 25° C.

	COEFFICIENT OF VISCOSITY IN DYNE-SECONDS PER CM.²	SPECIFIC VISCOSITY BASED ON WATER AT 25° C.
Water	0.009	1
Ethyl esters of olive oil	0.0092	1.02
Campiodol emulsion	0.0189	2.1
Rapeseed oil	0.045	5
Olive oil	0.045	5
Campiodol (4:1)	0.183	20.3
Campiodol	0.669	74.3
Lipiodol (iodized poppy seed oil)	0.438	48.6

According to the extensive laboratory and clinical researches of Frazier and Glaser,[5] the advantages of campiodol, in comparison with other iodized oils, in roentgenographic visualization, are mainly a result of its low toxicity, low viscosity, and its ability to cast excellent shadows. It has been shown experimentally that campiodol, whether diluted with ethyl olive oil, or not, is less toxic than a solution of sodium iodide containing the same quantity of iodine. Dogs have been given doses of 6.75 c.c. per kilo of body weight per os without any unfavorable reaction. For rabbits, the average maximum tolerated dose, introduced per os, is 1 gram per kilo, while the corresponding dose for lipiodol is 0.75 gm. per kilo of body weight of pure iodine.

Although any foreign substance introduced into the spinal subarachnoid space causes an increase in the cell count, campiodol gives the least reaction as compared with other iodized oils. The cell counts of eight dogs who received by cisternal puncture 2 c.c. of iodized oil varied from 250 to 800 per cubic millimeter. The highest count is usually on the second day, and after five or six days the cell count decreases to between 5 and 15. In the case of a patient with hydrocephalus, 7 c.c. of campiodol were injected into the ventricles; on the second day the cell count was 310 but decreased afterward. In a hydrocephalic baby who received intraventricularly 3.5 c.c. of the oil, the highest cell count was 19, three days later. Iodized rapeseed oil therefore causes a cellular reaction, but as can be seen it is mild and subsides rapidly.

Campiodol has been successfully used in neurologic visualization, in the diagnosis of various sinus infections, as well as in vascular, pulmonary, and genitourinary visualization.

Spinal block yields readily to diagnosis by means of campiodol.[6] The injection is made either by lumbar or cisternal puncture, and the flow is observed by reversing the position of the patient on the fluoroscopic table. Owing to the lack of globulation and the free flowing properties of campiodol, it does not adhere to the spinal roots, as is sometimes the case with other iodized oils, thus excluding the possibility of the so-called false block.

In vascular visualization, the use of a material irritating in the least degree may lead to severe complications, such as thrombosis and gangrene. The nonirritating nature of campiodol, however, allows it to be used for visualization of cranial and peripheral vessels; as much as 5 c.c. having been injected without any undesirable symptoms. Fat embolism has never been observed

in experimental work with campiodol. It has distinct possibilities in the field of vascular photography.

It has been found that best results in the visualization of the subarachnoid spaces, for vascular photography, fistula, mastoid, eustachian tubes, and in outlining the female genitalia, are obtained by mixing four parts of campiodol with one part of ethyl olive oil. Satisfactory shadows are obtained with this mixture, which can be readily injected, the ethyl olive oil tending to protect the campiodol from deterioration, without any increase in toxicity.

In sinus work,[7] a diminished shadow-casting ability is required, since great opacity would obscure important soft tissue details. A mixture of 50 per cent campiodol and 50 per cent ethyl olive oil has been found to be the most satisfactory combination, its low viscosity allowing it to be injected without special syringes, and permitting the oil to shift in the sinuses, which gives a better outline of the sinus walls.

For urographic purposes,[8, 9] ordinary iodized oils are unsuitable, owing to their high viscosity and nonmiscibility with urine. In an attempt to eliminate these disadvantages, emulsified campiodol was developed. Emulsified campiodol, prepared with acacia as emulsifying agent, is a stable, grayish-white oil emulsion, of specific gravity 1.078 and viscosity slightly more than twice that of water. The emulsion, if properly prepared, can be diluted with water without any separation of the oil. Emulsified campiodol gives excellent shadows in solutions containing from 7 to 10 per cent of iodine. This preparation mixes readily with urine and therefore allows a clear definition of every detail of the urinary tract. Its further advantage is its nonirritability; 5 and 12.5 per cent solutions of sodium iodide, on the other hand, nearly always produce pain, burning, urgency, and irritation.

In intrathoracic disease, campiodol may sometimes be the only means of accurate diagnosis and localization, since thickened pleura, pulmonary fibrosis, or emphysema may make physical and x-ray examinations ineffectual. Campiodol is in general useful whenever there is doubt as to the correct interpretation of symptoms and x-ray shadows. It has been successfully used in cases of chronic bronchitis, bronchiectasis, pulmonary abscess, bronchial fistula, bronchostenosis, tumors of the lung and mediastinum, and hernias of the diaphragm.

The wide range of application of campiodol for purposes of visualization is made possible by the fact that it combines the properties of an entirely inert substance with the ability to cast excellent shadows. These advantages result from its high iodine content, its stability, and the absence of irritative acids in rapeseed oil.

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THE KIDNEY THRESHOLD FOR GLUCOSE IN DIABETIC AND NONDIABETIC PERSONS*

By B. Y. Glassberg, M.D., St. Louis, Mo.

IT HAS been my experience in a large diabetic clinic that many patients are referred with a probable diagnosis of diabetes mellitus because a physician has found sugar in one or several specimens of urine. If this be true in a rather extensive clinic contact with diabetic patients, it must also be true that a large number of patients are being treated for diabetes by a private physician because of glycosuria. In view of the rigid dietary limitations imposed on diabetic patients, it seemed worth while to study anew the renal threshold for sugar, particularly in its relationship to the diagnosis of this serious disturbance in carbohydrate metabolism. The common refusal of life insurance companies to issue policies to applicants with glycosuria is too well known to require comment.

In this country clinicians generally hold the view that there is a fixed kidney threshold for glucose and that the elimination of sugar automatically requires a diagnosis of a disturbance in carbohydrate metabolism. Tachau[1] expressed such a view in 1911. In this country Hamman and Hirschman[2] reported that the threshold normally was between 170 and 180 mg. per cent. Their statement that it might lie as low as 140 mg. per cent seems to have been generally overlooked. Faber[3] further emphasized this misconception by reporting that the normal threshold was about 180 mg. per cent. The fallacy of considering glycosuria as evidence of a disturbed carbohydrate metabolism is strikingly emphasized by Holst's[4] analysis of the subsequent course of 150 persons refused life insurance on account of glycosuria. Of this number only 30 per cent had developed diabetes mellitus in the course of five to sixteen years. In other words the glycosuria in the remaining 70 per cent must be considered as a not abnormal finding. Eisner and Forster[5] in a curious report state that after a meal of white bread the blood sugar might go as high as 301 mg. per cent without the excretion of sugar but that after 100 gm. glucose, glycosuria occurred with a blood sugar of only 186 mg. per cent. Jacobsen[6] made a similar observation.

*From the Medical Service, Jewish Hospital.
Received for publication, February 3, 1931.

Arndt[7] found glycosuria in only a small number of 131 insane persons to whom he gave 100 gm. glucose; no blood-sugar studies were made. Liefman and Stern[8] on the basis of only a single analysis in each of eight persons assumed the threshold to lie between 105 and 224 mg. per cent. The results of Williams,[9] Faber and Hansen,[10] Hatlehol,[11] Wordley,[12] Rabinowitch,[13] the Goldbergers,[14] Lawrence,[15] and John,[16] may be summarized in the statement that they found the threshold as low as 80 and as high as 300 mg. per cent in both normal and diabetic patients. Goto and Kuno[17] found the threshold between 114 and 231 mg. per cent in 53 normal adults (Japanese). In studying 44 normal patients under ether anesthesia, Mackay[18] found glycosuria in one patient with a blood sugar of 133 mg. per cent while in another it had not occurred with a blood sugar of 386 mg. per cent. Hatlehol[19] in contrast to most authors, considers the threshold to be independent of age in diabetics. Faber[20] assumes that it is fixed for any one person while Chabanier and Lebert[21] found it fixed in some persons while shifting considerably in others.

In general, in these older methods venous blood was used for analysis although some authors fail to state the source of the blood and the method of analysis. Hence their conception fails to take into consideration the considerable arteriovenous difference in sugar content at the height of the curve (Glassberg[22]). In some cases this amounted to 60 mg. per cent. It must be evident that these earlier studies have been based upon the concentration at which sugar in the blood left the kidney rather than the concentration at which it reached the kidney and that for this reason a considerable error is inherent to the method. Another error in these older determinations has been avoided by use of the new analytical method of Somogyi[23] who showed that the true or fermentable blood sugar is 10 to 40 mg. per cent lower than that obtained by several of the older methods. This finding has never before been mentioned in threshold determinations. It is in these two respects, the use of capillary blood and the determination of true blood sugar, that the chief importance of this study lies.

I have examined several hundred sugar-tolerance tests made on capillary blood in the Jewish Hospital with the object of determining the kidney threshold for glucose. The threshold was taken at that point on the rising limb of the curve at which glycosuria just occurred. In those cases in which there was a considerable difference between two successive blood and urine sugar determinations, the curve was discarded because of the impossibility of telling just where the threshold lay.[10] Glycosuria when noted in the fasting specimen, was determined on a specimen voided after discarding the urine secreted for at least twelve hours after the last food. Where no glycosuria was found, the figure in the table represents the highest point on the curve. Cases in which the maximal blood sugar was below 175 mg. per cent are not included. Some of the determinations were made with the old tungstic acid method of precipitating blood proteins while the later ones are by Somogyi's method. Since 20 mg. per cent represents the average difference between tungstic acid and the zinc precipitation values of

blood sugar, this number was subtracted from the result of the older method in order to have comparable figures.

In Table I are recorded, the findings in 108 tolerance tests on diabetic and nondiabetic persons all above the age of seventeen. The practical equality in the number of the curves in each group must be taken as a coincidence rather than as a fact to which diagnostic value can be attached. The most important information to be gleaned from the table is that eight persons who showed no detectable disturbance in carbohydrate metabolism had enough sugar in the urine to be detected by the ordinary clinical test, while the blood sugar was below 99 mg. per cent. On the other hand 21 similar normal patients excreted no sugar into the urine despite a true blood sugar over 175 mg. per cent. The threshold in the diabetic was just as variable as in the nondiabetic. It is thus apparent that the kidney threshold for glucose may be below 100 or above 250 mg. per cent; it appears to be independent of both age and sex.

TABLE I

TRUE SUGAR IN MG. PER 100 C.C. CAPILLARY (ARTERIAL) BLOOD	NUMBER OF CASES IN WHICH GLYCOSURIA DID NOT OCCUR AT THIS BLOOD-SUGAR LEVEL		NUMBER OF CASES IN WHICH GLYCOSURIA JUST OCCURRED AT THIS BLOOD-SUGAR LEVEL		NUMBER OF PATIENTS
	NONDIABETIC	DIABETIC	NONDIABETIC	DIABETIC	
Above 250 mg. per cent	2	1	0	13	16
249 to 225 mg. per cent	2	2	2	7	13
224 to 200 mg. per cent	7	2	4	3	16
199 to 175 mg. per cent	10	5	7	6	28
174 to 150 mg. per cent			5	5	10
149 to 125 mg. per cent			3	6	9
124 to 100 mg. per cent			1	4	5
Below 99 mg. per cent			8	3	11
Total Cases	21	10	30	47	108

Table I shows the level above the commonly accepted renal threshold for glucose reached without glycosuria in nondiabetics and diabetics, and the level at which glycosuria (judged by Benedict's method) just occurred. Cases of renal glycosuria are not included. The true sugar is 20 mg. per cent lower than that obtained by the older methods.

The Benedict method commonly used in this country as a means of determining glycosuria was used throughout. Normal glycuresis gives no reaction by this method. In a few cases in which the accuracy of the determination was in doubt, the test was confirmed by Somogyi's[24] yeast method; a finding of considerable interest in the case of two normal women who had volunteered for study. Reduction of Benedict's solution by their fasting urine but not their later urine was proved to be due to fermentable sugar by the yeast test.

This finding emphasizes another fact not brought out by the table: the inconstancy of the threshold for glucose in the same individual, as shown either on repetition of the test or even during one tolerance estimation. For example, in a forty-five-year-old diabetic patient glycosuria was demonstrated with a blood sugar of 98 mg. per cent; a year later there was no glycosuria during the course of a tolerance test until the blood sugar was over 200 mg. per cent. In a twenty-two-year-old nondiabetic patient glycosuria was pres-

ent with a fasting blood sugar of 85 mg. per cent although it had not occurred in a test done two days earlier at a blood-sugar level of 172 mg. per cent.

The experiments by which Cannon[25] induced glycosuria by frightening cats are well known. Less generally known is the fact that glycosuria may be induced by mechanical methods other than the piqûre of Claude Bernard, notably by vagus stimulation (Hildebrandt[26]), by partial axphyxia with increase in the carbon dioxide content of the blood (Eadie and others[27]), and by such commonly used drugs as sodium salicylate (Graham[28]). These findings are all in keeping with the dictum of von Jaksch[29] and many others, that glycosuria is a prominent symptom of diabetes mellitus rather than the disease itself.

CONCLUSIONS

1. Contrary to the generally accepted impression, the kidney threshold for glucose shows wide individual differences. It may be below 100 or above 250 mg. per cent true blood glucose. It appears independent of age and sex.

2. The kidney threshold for glucose is not fixed in any one person and may show marked shifts within the space of a few minutes.

3. Glycosuria in the absence of other findings does not indicate a disturbance in carbohydrate metabolism. The diagnosis of diabetes mellitus cannot be made on the mere presence of sugar in the urine. It cannot always be made in the presence of a high blood sugar and glycosuria.

4. In order to prevent unjustified dietary limitation, careful study is required before making a diagnosis of diabetes mellitus.

5. Life insurance companies may safely revise their requirements so as not to exclude indiscriminately, persons whose urine reveals a glycosuria.

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A STUDY OF THE VISCOSITY OF BLOOD SERUM IN SYPHILIS, WITH A REPORT ON THE SERUM ALBUMIN AND SERUM GLOBULIN CONTENT*

By Mary C. McIntyre, M.D., Kalamazoo, Mich.

ACCORDING to Bircher[1] and Van der Zee,[2] viscosity of the blood and serum has not received the consideration it should have in the general diagnosis and study of hemodynamics.

The literature on viscosity per se is extensive but that which deals with viscosity in disease is not quite so large. Of those reports published dealing with viscosity only those will be cited that have a bearing on the subject discussed here.

Poisieuille's law states that fluids at equal temperature and equal pressure, passing through capillary tubes of equal caliber, vary in their rate of flow in direct proportion to their internal friction, i.e., to their viscosity.

Naegeli reported the normal viscosity as ranging from 1.7 to 2.0; Bircher[3] studied 14 cases where the viscosity of the serum was found to be 1.61 to 1.68; Bircher and McFarland,[5] using serum from patients with primary and secondary syphilis reported the viscosity to be 1.86; in tertiary syphilis they reported 1.83; in central nervous system syphilis 1.82; and in patients with untreated syphilis, it was perhaps a little higher but of no great significance. They could find no relation between the clinical types of syphilis and viscosity and concluded that viscosity of itself is of little significance either as an aid to diagnosis or to the differentiation of clinical types.

*From the Department of Dermatology and Syphilis, University of Pennsylvania, Dr. John H. Stokes, Director.

Received for publication, January 19, 1931.

Bircher and McFarland[4] state that an increase of globulin is not specific for any disease but that there may be a quantitative relationship to pathologic processes. Syphilitic serum has been found to have an increase in its globulin content which is associated with immunization and is decreased by arsphenamine. The normal content of albumin is 60 to 80 per cent and that of globulin is 20 to 40 per cent. In the 14 cases studied by Bircher and McFarland,[4] the albumin was 59 to 63 per cent and globulin 37 to 41 per cent. These authors tabulated the observations of various authors giving 22 to 39 per cent for globulin with outside limits as 11 to 47 per cent; for globulin the average ranged from 61 to 88 per cent with limits of 53 to 89 per cent.

Of their own cases 90 per cent showed a globulin content of 50 per cent and a great number were observed to decrease with treatment. They believe that the globulin content is more constant and less subject to fluctuation than serologic tests and that it gradually decreases as treatment progresses.

Technic.—For viscosimetric determinations the Hess viscosimeter, fully described by Bircher,[1] was used. DuNuoy[5] studied viscosity as a function of time and found that determinations made immediately after withdrawal of blood had a higher viscosity (2.640) than that allowed to stand for a period varying from forty-five minutes to one hour. Those allowed to stand for this period gave readings which became lower until at the end of one hour they reached a point where stabilization occurred.

The method of Wu and Ling[6] was used for the determination of the serum albumin and the serum globulin.

Cases Studied.—Sera was obtained from proved syphilitic and proved non-syphilitic patients. Every case had a complete physical examination, neurologic examination, serologic tests repeated several times, spinal fluid examination, and such others as were indicated in special fields. The diagnosis in every case was based on a complete study to establish or negate the diagnosis of syphilis.

There were 376 cases studied with 1,022 viscosimetric determinations. There were 82 cases of primary and secondary syphilis, 34 late cutaneous syphilis, 20 central nervous system syphilis, 16 heredosyphilis, 44 latent syphilis, 15 investigations on cases of doubtful syphilis, and 165 miscellaneous dermatoses.

Of the 65 cases studied for serum albumin and globulin content 44 gave the usual rise in globulin and will not be discussed further. There were 21, however, that did not show this rise and they will be outlined later.

Results in Viscosimetric Determination.—Table I shows the division of cases into the various types of syphilis, the number of cases in each type, the total number of viscosimetric readings made, the serologic results, and the average viscosity reading for each type of case.

On the whole both the Kolmer modification of the Wassermann test and the Kahn test were positive where the viscosity was increased. The Kolmer modification of the Wassermann test gave 7 negative reactions and the Kahn precipitation test 22 negative results when the viscosity was increased as is seen in Table I. The larger number of disagreements between the serology

and viscosity are to be found in primary and secondary syphilis and, further, in those cases of primary syphilis where the serology later became positive.

The average viscosity in primary and secondary syphilis as observed in this work was 1.99 whereas Bircher and McFarland[5] reported it to be 1.86. In central nervous system syphilis the viscosity reported here parallels theirs.

In 13 instances it was noted that during the course of treatment, the serologic test would occasionally give a negative reaction following a series of positive tests and then be followed again by positive serologic reactions. The viscosity at the time of the negative test would remain increased. It was also noted that the viscosity would show an inclination to decrease before the serologic test became negative and this decrease in viscosity would be followed by a definite tendency of the serology toward a negative reaction.

TABLE I

	NO. OF CASES	NO. OF DETERMINATIONS	KOLMER POS.	KOLMER NEG.	KAHN POS.	KAHN NEG.	AVERAGE VISCOSITY
Primary and Secondary Syphilis	82	424	420	4	416	8	1.99
Late Cutaneous Syphilis	34	86	86	0	82	4	1.87
Latent Syphilis	44	156	154	2	153	3	1.70
Central Nervous System Syphilis	20	71	70	1	67	4	1.82
Heredosyphilis	16	49	49	0	46	3	1.74
Nonspecific Dermatoses	165	205	5	200	5	200	1.60

Table II represents only those cases having 6 or more viscosimetric determinations at weekly intervals along with the serologic results on the same serum. This table supplies the following observations: (1) Negative serology preceded and followed by a series of positive results in which the viscosity did not vary from its increase as in Cases 1, 2, 4, 6, 9, 10, 13, 14, 15, and 16. (2) Viscosity that had begun to decrease before the serology showed a negative tendency as in Cases 3, 11, and 12. (3) A combination of (1) and (2): as in Case 5. (4) Serologic tests negative and viscosity increased at onset followed by a period when the serologic tests became positive and the viscosity remained increased with a final decline in both as in Case 7. (5) Both viscosity and serologic tests parallel each other as in Case 8.

Serum Albumin and Serum Globulin.—Sixty-five cases were studied for serum albumin and serum globulin content of serum in relation to viscosity. In 21 of the 65 cases the increased viscosity was not due to an increased serum globulin; the other 43 cases showed an increase of serum globulin and will not be discussed or tabulated. The results in the 21 cases where the serum globulin was not increased are tabulated in Table III.

The viscosimetric readings ranged from 1.81 to 2.01, the albumin from 61 to 80 per cent, and the globulin from 20 to 39 per cent. The serum albumin and globulin content remained within the limits given as normal while to viscosity was increased.

It may be that a colloidal state of the albumin might be the cause of the increased viscosity but the number of cases studied numbered but 65, therefore no definite conclusions can be drawn. The findings, however, indicate

TABLE III

Case Number	Diagnosis	Viscosimetric Reading No. 1			2			3			4			5			6			7			8			9			10			11			12			13			14			15		
		Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity	Kolmer	Kahn	Viscosity
1	Primary Syphilis	44	000	1.91	44	012	2.1	44	444	2.1	44	122	1.99	44	111	1.97	00	012	1.95	44	444	1.94	33	444	1.91	00	000	1.9	44	122	1.91	00	000	1.9	33	233	1.91	44	333	1.9						
2	Primary Syphilis	44	444	1.99	00	000	2.0	44	444	2.0	44	444	1.91	43	344	1.89	23	344	1.89	44	444	1.89	44	444	1.89	44	441	1.89	44	233	1.87	44	444	1.87											
3	Primary Syphilis	44	444	2.06	44	233	2.02	44	344	2.0	34	444	2.0	44	122	2.0	44	444	1.87	00	000	1.81	00	122	1.8	21	333	1.8	12	012	1.77	00	000	1.7	00	012	1.68	00	000	1.65	00	000	1.64			
4	Secondary Syphilis	44	443	1.92	44	444	2.0	44	444	1.86	00	000	1.86	00	000	1.81	43	444	1.9	44	444	1.9	41	344	1.9	00	000	1.87	44	444	1.89														
5	Primary Syphilis	44	233	1.97	12	233	1.9	00	000	1.91	44	444	1.9	00	000	1.87	44	444	1.88	*	-2	1.84	01	012	1.82	00	012	1.79																		
6	Primary Syphilis	44	444	2.0	44	444	2.0	44	444	1.99	44	444	1.96	00	222	1.98	44	444	1.99	44	444	1.98	44	444	1.97	44	444	2.0																		
7	Primary Syphilis	00	122	1.9	44	444	1.91	44	444	1.91	44	444	1.9	24	122	1.89	00	000	1.89	44	122	1.89	22	122	1.86																				
8	Secondary Syphilis	44	444	2.1	44	444	2.1	44	444	2.0	44	444	1.98	44	444	1.97	00	001	1.92	01	122	1.82	00	000	1.79	00	000	1.71																		
9	Primary Syphilis	00	000	1.92	34	244	1.96	44	344	1.97	00	011	1.8	00	122	1.9	43	122	1.88	00	111	1.8	00	122	1.8	00	000	1.81	43	122	1.8	00	000	1.8	00	000	1.75	00	000	1.71	00	000	1.64	00	000	1.65
10	Primary Syphilis	44	444	2.02	44	444	1.97	32	111	1.93	00	000	1.9	00	000	1.89	43	233	1.89	43	122	1.9	44	444	1.9	44	233	1.88	32	122	1.86														
11	Secondary Syphilis	44	444	2.02	44	444	2.0	44	444	1.97	34	444	1.89	00	000	1.8	44	444	1.8	00	000	1.7	00	000	1.65	00	00	1.61	11	111	1.6	00	000	1.6											
12	Primary Syphilis	44	444	2.0	44	444	1.9	44	444	1.99	44	444	1.9	44	444	1.85	23	222	1.8	00	001	1.76	00	000	1.7	00	000	1.65																		
13	Late Cutaneous Syphilis	44	444	2.0	44	444	2.0	*	122	2.0	44	444	2.0	44	444	2.0	44	444	2.0																										
14	Heredo Syphilis	44	344	2.0	44	444	1.89	01	000	1.85	44	444	1.92	44	444	1.93	44	444	1.94																										
15	Central Nervous System Syphilis	44	444	2.0	00	000	2.0	44	443	1.99	44	444	1.99	44	444	1.99	44	444	1.99	44	444	1.99	44	233	1.99																				
16	Latent Syphilis	00	000	1.79	00	000	1.81	00	000	1.89	44	444	1.89	44	444	1.89	34	122	1.85	00	000	1.89	44	441	1.95																				

*± ±

that further work might show a relation between the rise of serum albumin and an increased viscosity. The increase in the latter has heretofore, been attributed to the increased globulin content of the serum.

TABLE III

NUMBER	DIAGNOSIS	KOLMER	KAHN	SERUM ALBUMIN PER CENT	SERUM GLOBULIN PER CENT	VISCOSITY
1	Primary syphilis	44	444	68	32	1.91
2	Primary syphilis	43	000	71	29	1.83
3	Primary syphilis	33	222	61	39	1.87
4	Secondary syphilis	44	322	72	28	1.92
5	Secondary syphilis	44	444	79	21	1.92
6	Secondary syphilis	43	322	67	33	1.9
7	Secondary syphilis	44	443	80	20	1.92
8	Secondary syphilis	44	333	75	25	1.89
9	Latent syphilis	33	333	67	33	1.82
10	Latent syphilis	44	000	69	31	1.85
11	Latent syphilis	34	111	71	29	1.81
12	C. N. S. syphilis	43	222	68	32	1.89
13	C. N. S. syphilis	44	444	75	25	1.91
14	C. N. S. syphilis	34	322	69	31	1.96
15	Late cutaneous syphilis	44	444	76	24	1.83
16	Late cutaneous syphilis	33	222	69	31	1.81
17	Late cutaneous syphilis	44	433	74	26	2.01
18	Heredo-syphilis	44	444	68	32	1.87
19	Heredo-syphilis	43	111	76	24	1.86
20	Heredo-syphilis	42	332	78	22	1.89
21	Cardiovascular syphilis	33	111	69	31	1.87

CONCLUSIONS

1. We agree with the statement by Bircher and Van der Zee that further work on serum viscosity in its relation to syphilis and serologic tests is needed.

2. With the exception of cases of primary and secondary syphilis our viscosimetric results agree with Bircher and McFarland. They found the viscosity in central nervous system syphilis to be 1.82 as we did. In primary and secondary syphilis our viscosimetric readings were 1.99 and theirs 1.86.

3. Viscosity will not, per se, diagnose syphilis or differentiate the type of syphilis but it may on fuller study supplement physical, serologic, and spinal fluid examinations.

4. Increased globulin alone does not explain the increased viscosity we noted. A study of the colloidal state of the serum albumin as the cause of increased viscosity would be worthy of investigation.

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A STUDY OF THE ANTIBACTERIAL PROPERTIES OF PYRIDIUM, URINARY ANTISEPTIC

By Alfred Goerner,* M.D., and Frank L. Haley,* M.D., Brooklyn, N. Y.

THE use of aniline dyes in medicine has become quite general, especially during the past few years.

Dyes are no longer utilized exclusively as stains or indicators in microscopic and diagnostic technic but have found a permanent place in therapeutics, mostly as antiseptics. And, they continue to play a very important rôle in biochemical research, without which Ehrlich and those who followed him could not have developed and perfected modern chemotherapy.

As antiseptics, dyes owe their therapeutic value to their selective bacteriostatic power and to their keratoplastic action. That is to say, they have a discriminative affinity for the particular species of organism responsible for a given infection as well as discriminative power to stimulate the growth of epithelial tissue. These parasitotropic and organotropic properties not only prevent or arrest the destructive action of the organisms which cause the infection without injuring the tissues of the host, but actually excite or promote the growth of the microbe-damaged tissue itself.

Some dyes are especially effective against gram-positive, others against gram-negative organisms, and certain dyes combat protozoa more readily than bacteria. From the great variety of chemical substances used as antiseptics, it is obvious that there may be many modes of action. In each case there may be a direct action on the organism involved, but this need not be the only mode of action, since some which are effective in destroying bacteria in the body will not do so in the same concentration in vitro. This is especially true of dyes. In such cases the dye-antiseptic causes changes in the tissues of the host which renders these less favorable for or more active against the life of the organism.

Recently a new dye has been introduced especially for internal use as a urinary antiseptic. It seems to have a decided advantage over the older products inasmuch as its oral administration even over a comparatively long period is not attended by any systemic by-effects.

The present studies concern only our bacteriologic findings of this new dye-antiseptic known as pyridium, which is chemically identified as phenyl-azo-alpha-alpha-diamino pyridine hydrochloride.

In this series of tests we are concerned only with the action of pyridium on those pathogenic organisms which are the ordinary cause of urinary infection and in relatively determining its bacteriostatic and bactericidal power in vitro so that the practicing physician will have a reliable guide as to the extent of its usefulness in the various clinical manifestations of urinary infection.

The tests performed to establish its antibacterial properties were as follows:

*Associate Professors of Biological Chemistry, Long Island College Hospital.
Received for publication, August 19, 1930.

We first undertook to determine the efficiency of pyridium on such organisms as B. coli communis, Staphylococcus pyogenes aureus, Klebs-Loeffler bacillus, Diplococcus pneumoniae and streptococcus (Gay).

The so-called tube method was used in this investigation.

TECHNIC OF THE TUBE METHOD

The tube method is a modification of Kolmer's bacteriostatic and bactericidal methods. We used nutrient broths and broths containing urine and serum, as well as hormone broth as culture media.

A stock solution of pyridium (1:200) was prepared. This solution was diluted with sterile distilled water so as to give final concentrations of 1:2,000, 1:3,000, etc., after we had added a broth suspension of the various organisms.

The suspension of organisms was made by taking 99 c.c. of sterile nutrient broth and adding 1 c.c. of a forty-eight-hour culture of the test organism. Nine c.c. of the broth suspension of organism was added to 1 c.c. of each of the several dilutions of pyridium, giving a final volume of 10 c.c. in each tube. The tubes were incubated for five days at 37.5° C. The results were recorded daily. A clear medium or one which exhibited no visible growth was recorded by a minus sign and a visible growth by a plus sign. At the expiration of five days subcultures were made by transferring 0.1 c.c. from each tube to flasks containing 100 c.c. of nutrient broth of the same P_H as the original tube. Controls were made by taking 10 c.c. of the broth suspension of the organisms. Subcultures were made from the controls by taking 0.1 c.c. of the control media and adding it to 100 c.c. of nutrient broth.

In each series of experiments the type of media used and the P_H is given in the table.

TABLE I

RESULTS WITH B. COLI

	MEDIUM = NUTRIENT BROTH. P_H 7.0					
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	–	–	–	–	–	–
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	+	+
1:7000	–	–	–	+	+	+
1:8000	–	–	–	+	+	+
Control	+	+	+	+	+	+

The results obtained with B. coli indicate that pyridium is bacteriostatic in as high a dilution as 1:8000 for a period of three days, and bactericidal up to 1:5000.

TABLE II

RESULTS WITH STAPHYLOCOCCUS PYOGENES AUREUS

	MEDIUM = NUTRIENT BROTH. P_H 7.0					
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	–	–	–	–	–	–
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	–	+
1:8000	–	–	–	–	+	+
Control	+	+	+	+	+	+

In the case of Staphylococcus pyogenes aureus, pyridium is bacteriostatic up to the fifth day in a dilution of 1:8000, and bactericidal up to 1:5000.

TABLE III

RESULTS WITH STREPTOCOCCUS (GAY)

MEDIUM = NUTRIENT BROTH. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	–	–	–	–	–	–
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	+
1:6000	–	–	–	–	–	+
1:7000	–	–	–	–	–	+
1:8000	–	–	–	–	–	+
1:10,000	–	–	–	–	–	+
Control	+	+	+	+	+	+

Pyridium was found to be bacteriostatic for this organism in dilutions as high as 1:10,000 and bactericidal up to 1:4000.

TABLE IV

RESULTS WITH KLEBS-LOEFFLER BACILLUS

MEDIUM = NUTRIENT BROTH. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	–	–	–	–	–	–
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	–	+
1:8000	–	–	–	–	–	+
1:10,000	–	–	–	–	–	+
Control	+	+	+	+	+	+

As a bacteriostatic agent pyridium was found to be effective in dilution up to 1:10,000 and bactericidal up to 1:5000, against Klebs-Loeffler Bacillus.

TABLE V

RESULTS WITH PNEUMOCOCCUS

MEDIUM = HORMONE BROTH. P_H 7.0						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	–	–	–	–	–	–
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	–	–
1:8000	–	–	–	–	–	–
1:10,000	–	–	–	–	–	–
Control	+	+	+	+	+	+

Pyridium in the case of pneumococcus was found to be bacteriostatic and bactericidal in dilutions up to 1:10,000.

SUMMARY OF THE FIRST SERIES

In the first series of experiments the P_H varied from 6.8 to 7.0.

In the cases in which nutrient broth was the medium used the bactericidal power varied 1:4,000 with streptococcus Gay to 1:5,000 with B. coli, Staphylococcus pyogenes aureus and Klebs-Loeffler bacillus. The bacteriostatic range varied from 1:8,000 on the fourth day with B. coli up to 1:10,000 with Streptococcus, Staphylococcus pyogenes aureus and Klebs-Loeffler Bacillus.

TABLE VI

RESULTS WITH B. COLI

MEDIUM = NUTRIENT BROTH + STERILE URINE. P_H 6.6						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	+	+
1:7000	–	–	–	–	+	+
1:8000	–	–	–	–	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic agent in dilutions of 1:8000 up to the fifth day and bactericidal up to 1:5000, against B. coli.

TABLE VII

RESULTS WITH STAPHYLOCOCCUS PYOGENES AUREUS

MEDIUM = NUTRIENT BROTH + 20 PER CENT URINE. P_H 6.6						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	+	+
1:7000	–	–	–	–	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic agent in dilutions as high as 1:7000 up to the fifth day and bactericidal up to 1:5000, against Staphylococcus pyogenes aureus.

TABLE VIII

RESULTS WITH STREPTOCOCCUS GAY

MEDIUM = NUTRIENT BROTH + 20 PER CENT URINE. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	+	+
1:7000	–	–	–	–	+	+
1:10,000	+	+	+	+	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic in dilutions as high as 1:7000 up to the fourth day and bactericidal up to 1:5000, against Streptococcus Gay.

TABLE IX

RESULTS WITH KLEBS-LOEFFLER BACILLUS

MEDIUM = NUTRIENT BROTH + 20 PER CENT URINE. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	+
1:6000	–	–	–	–	–	+
1:7000	–	–	–	–	–	+
1:8000	–	–	–	–	+	+
1:10,000	–	+	+	+	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic in dilutions of 1:8000 up to the fifth day and bactericidal up to 1:4000, against Klebs-Loeffler Bacillus.

TABLE X

RESULTS WITH PNEUMOCOCCUS

MEDIUM = NUTRIENT BROTH + 20 PER CENT URINE. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	–	–
1:7000	–	–	–	–	–	+
1:8000	–	–	–	–	+	+
1:10,000	–	–	–	+	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic in dilutions of 1:10,000 up to the fourth day and bactericidal up to 1:6000.

In the experiments in which hormone broth was used the bacteriostatic and bactericidal limit was 1:10,000.

A second series of tests was made in which the preceding technic was followed with the exception that 20 per cent sterile urine was added to the nutrient broth used in obtaining a suspension of the organism.

SUMMARY OF THE SECOND SERIES OF TESTS

In the second series of tests in which 20 per cent urine was added to the nutrient broth the P_H varied from 6.6 to 6.8. The bacteriostatic power varied 1:7,000 with Staphylococcus pyogenes aureus at the fourth day to 1:8,000 at

TABLE XI

RESULTS WITH B. COLI

MEDIUM = NUTRIENT BROTH + 20 PER CENT NORMAL HORSE SERUM. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	–	+
1:7000	–	–	–	–	+	+
1:8000	–	–	–	+	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic in dilutions of 1:8000 up to the fourth day and bactericidal up to 1:5000, against B. coli.

TABLE XII

RESULTS WITH STAPHYLOCOCCUS PYOGENES AUREUS

MEDIUM = NUTRIENT BROTH + 20 PER CENT NORMAL HORSE SERUM. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	–	+
1:7000	–	–	–	+	+	+
1:8000	–	–	+	+	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic in dilutions of 1:8000 up to the third day and bactericidal up to 1:5000, against Staphylococcus pyogenes aureus.

the fourth day in the cases of B. coli, Streptococcus (Gay) and Klebs-Loeffler Bacillus and 1:10,000 at the fourth day with pneumococcus. The bactericidal power varied from 1:4,000 in the case of Klebs-Loeffler Bacillus to 1:6,000 in the case of pneumococcus.

A third series of experiments was conducted using a nutrient broth to which 20 per cent horse serum was added in making a suspension of the organisms.

TABLE XIII

RESULTS WITH STREPTOCOCCUS (GAY)

MEDIUM = NUTRIENT BROTH + 20 PER CENT NORMAL HORSE SERUM. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	–	+	+
1:7000	–	–	–	+	+	+
1:8000	–	–	–	+	+	+
1:10,000	–	–	+	+	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic in dilutions of 1:10,000 up to the third day and bactericidal up to 1:5000, against streptococcus (Gay).

TABLE XIV

RESULTS WITH KLEBS-LOEFFLER BACILLUS

MEDIUM = NUTRIENT BROTH + 20 PER CENT NORMAL HORSE SERUM. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	+
1:6000	–	–	–	–	+	+
1:7000	–	–	–	+	+	+
1:8000	–	–	+	+	+	+
1:10,000	–	+	+	+	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic in dilutions of 1:10,000 up to the second day and bactericidal up to 1:4000, against Klebs-Loeffler bacillus.

TABLE XV

RESULTS WITH PNEUMOCOCCUS

MEDIUM = NUTRIENT BROTH + 20 PER CENT NORMAL HORSE SERUM. P_H 6.8						
DILUTION	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	SUBCULTURE
1:2000	ppt.	ppt.	ppt.	ppt.	ppt.	Not done
1:3000	–	–	–	–	–	–
1:4000	–	–	–	–	–	–
1:5000	–	–	–	–	–	–
1:6000	–	–	–	+	+	+
1:7000	–	–	–	+	+	+
1:8000	–	–	–	+	+	+
1:10,000	–	–	+	+	+	+
Control	+	+	+	+	+	+

Pyridium was effective as a bacteriostatic in dilutions of 1:10,000 up to the third day and bactericidal up to 1:5000.

SUMMARY OF THE THIRD SERIES

In the third series of experiments in which 20 per cent normal horse serum was used all the P_H values were 6.8.

The bacteriostatic power varied from 1:5,000 for Klebs-Loeffler bacillus at the fifth day to 1:6,000 for B. coli and Staphylococcus pyogenes aureus at the fifth day.

The bactericidal power varied from 1:4,000 for Klebs-Loeffler bacillus to 1:5,000 for pneumococcus, B. coli, Staphylococcus pyogenes aureus, and streptococcus (Gay).

FOURTH SERIES OF EXPERIMENTS

A fourth series of experiments was conducted in accordance with the agar plate method which is as follows:

One hundred c.c. of liquefied nutrient agar containing 3 per cent agar, 1 per cent peptone, 0.6 per cent beef extract (Liebig) and having a definite P_H value. The mixture was brought to a temperature of 55° C. and then 20 per cent of normal horse serum was added. A suspension of the respective pathogenic organism was made, by mixing 0.5 c.c. of a twenty-four-hour broth culture with 99.5 c.c. of sterile water containing 0.5 per cent sodium chloride. All the pathogenic microorganisms used were transplanted daily for eight

TABLE XVI

RESULTS WITH B. COLI

MEDIUM = NUTRIENT AGAR PLUS 10 PER CENT NORMAL HORSE SERUM. P_H 6.8				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURES AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	–	–	–	+
1:8000	–	12	104	+
1:10,000	15	100	+	Not done
Control	uncount.	uncount.	uncount.	+

Using the agar plate technic pyridium was found to be bactericidal for B. Coli in dilutions up to 1:6000 and bacteriostatic in dilutions up to 1:8000 for twenty-four hours.

TABLE XVII

RESULTS WITH STAPHYLOCOCCUS PYOGENES AUREUS

MEDIUM = NUTRIENT AGAR PLUS 10 PER CENT NORMAL HORSE SERUM. P_H 6.8				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	–	–	–	–
1:8000	–	4	50	+
1:10,000	–	10	20	+
Control	uncount.	uncount.	uncount.	+

Under the conditions of this experiment with Staphylococcus pyogenes aureus pyridium was found to be bactericidal in dilutions up to 1:6000 and bacteriostatic in dilutions up to 1:10,000 for twenty-four hours.

days before the experiments. One c.c. of this suspension was mixed thoroughly with 100 c.c. of the agar, which resulted in a final even suspension of the bacteria, 1:20,000. Ten c.c. of this inoculated agar was mixed thoroughly with 10 c.c. of a warm solution of pyridium of such a concentration as to make the final concentration of the compound 1:2,000, 1:3,000, etc. Control plates were made by mixing 10 c.c. of sterile water with 10 c.c. of the inoculated agar. The nutrient media contained in its final dilution 1.5 per cent of agar, 10 per cent horse serum, 0.5 per cent peptone, and 0.3 per cent beef extract.

TABLE XVIII

DATA OBTAINED WITH STREPTOCOCCUS (GAY)

MEDIUM = HORMONE NUTRIENT AGAR + 10 PER CENT NORMAL HORSE SERUM. P_H 6.8				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	+
1:5000	–	–	–	+
1:6000	–	10	83	+
1:7000	–	18	103	+
1:8000	–	71	205	+
1:10,000	uncount.	uncount.	uncount.	uncount.
Control	+	+	+	+

With streptococcus (Gay) pyridium showed bactericidal action in dilutions up to 1:3000 and bacteriostatic action up to 1:10,000 for twenty-four hours.

TABLE XIX

DATA OBTAINED WITH KLEBS-LOEFFLER BACILLUS

HORMONE NUTRIENT AGAR PLUS 10 PER CENT NORMAL HORSE SERUM. P_H 6.8				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	–	9	43	+
1:7000	–	12	61	+
1:8000	–	10	209	+
1:10,000	47	uncount.	uncount.	+
Control	+	+	+	+

Pyridium with Klebs-Loeffler bacillus showed bactericidal action in dilutions up to 1:5000 and bacteriostatic up to 1:8000 for twenty-four hours.

TABLE XX

DATA OBTAINED WITH PNEUMOCOCCUS

HORMONE NUTRIENT AGAR + 10 PER CENT NORMAL HORSE SERUM. P_H 6.8				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	–	11	39	+
1:8000	8	25	112	+
1:10,000	17	38	292	+
Control	+	+	+	+

In the case of pneumococcus, pyridium showed bactericidal action in dilutions up to 1:5000 and bacteriostatic action up to 1:6000 for twenty-four hours.

The mixture was then poured into sterile Petri dishes and after solidification took place they were incubated at 37.5° C. Examinations with a magnifying glass were made for visible growth after twenty-four hours and after forty-eight hours.

Subcultures were made after forty-eight hours by picking out particles of 0.1 cm. in diameter from four different parts of the plate and transferring same into 100 c.c. of the nutrient broth, crushing with a sterile rod and incubating these subcultures for forty-eight hours at 37.5° C. The plates were kept in the incubator for another twenty-four hours and observed again, and the results noted.

FIFTH SERIES OF EXPERIMENTS

A fifth series of experiments was made using nutrient agar containing 10 per cent of sterile urine. The general technic was the same as that employed in the agar plate method described in the fourth series of experiments.

TABLE XXI

DATA OBTAINED WITH B. COLI

NUTRIENT AGAR PLUS 10 PER CENT STERILE URINE. P_H 6.6				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	–	–	–	+
1:8000	–	–	–	+
1:10,000	50	300	649	+
Control	uncount.	uncount.	uncount.	+

In the presence of urine pyridium is bactericidal for B. coli in dilutions up to 1:5000 and bacteriostatic up to 1:8000 for seventy-two hours.

TABLE XXII

DATA OBTAINED WITH STAPHYLOCOCCUS PYOGENES AUREUS

NUTRIENT AGAR PLUS 10 PER CENT STERILE URINE. P_H 6.6				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	–	–	–	+
1:8000	–	–	–	+
1:10,000	116	uncount.	uncount.	+
Control	uncount.	uncount.	uncount.	+

In the case of Staphylococcus pyogenes aureus, pyridium in the presence of urine is bactericidal in dilutions up to 1:5000 and bacteriostatic up to 1:8000 for seventy-two hours.

TABLE XXIII

DATA OBTAINED WITH STREPTOCOCCUS (GAY)

NUTRIENT AGAR PLUS 10 PER CENT STERILE URINE. P_H 6.8				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	–	–	–	–
1:8000	–	9	24	+
1:10,000	23	65	701	+
Control	uncount.	uncount.	uncount.	+

Pyridium in the presence of urine is bactericidal for Streptococcus (Gay) in dilutions up to 1:6000 and bacteriostatic up to 1:8000 for twenty-four hours.

TABLE XXIV

DATA OBTAINED WITH KLEBS-LOEFFLER BACILLUS

NUTRIENT AGAR PLUS 10 PER CENT STERILE URINE. P_H 6.8				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	–	15	68	+
1:8000	–	18	85	+
1:10,000	40	166	359	+
Control	uncount.	uncount.	uncount.	+

With Klebs-Loeffler bacillus in the presence of urine pyridium is bactericidal up to 1:5000 and bacteriostatic up to 1:8000 for twenty-four hours.

TABLE XXV

DATA OBTAINED WITH PNEUMOCOCCUS

NUTRIENT AGAR PLUS 10 PER CENT STERILE URINE. P_H 7.0				
DILUTION	24 HR.	48 HR.	72 HR.	SUBCULTURE AFTER 48 HR.
1:2000	–	–	–	–
1:3000	–	–	–	–
1:4000	–	–	–	–
1:5000	–	–	–	–
1:6000	7	13	42	+
1:8000	5	12	39	+
1:10,000	26	49	141	+
Control	uncount.	uncount.	uncount.	+

Pyridium in the presence of urine is bactericidal for pneumococcus in dilutions up to 1:5000. The bacteriostatic action is likewise 1:5000.

SUMMARY OF THE FIFTH SERIES

In this series the media consisted of nutrient agar plus 10 per cent sterile urine. The P_H varied from 6.6 to 7.0. The bactericidal power of pyridium under these conditions varied 1:5,000 for B. coli, Staphylococcus pyogenes aureus, Klebs-Loeffler bacillus and pneumococcus to 1:6,000 for streptococcus (Gay). The bacteriostatic power of pyridium varied from 13 colonies for pneumococcus at 1:6,000 for forty-eight hours to 300 colonies for B. coli at 1:10,000 for forty-eight hours.

SUMMARY AND FINAL CONCLUSIONS

Experiments in vitro indicate that the bactericidal action of pyridium under the conditions followed in the above experiments varies between 1:4,000 and 1:10,000. The average bactericidal power being about 1:5,000.

The bacteriostatic power varied from 1:4,000 to 1:10,000. The average in this case was about 1:8,000.

The use of a number of special media did not materially affect the results.

An attempt was made to ascertain whether a slight variation in P_H materially changed the findings. The results obtained would not seem to so indicate. Good growths were obtained in the controls with all the organisms, showing that slight differences in P_H did not appreciably affect the growth of the cultures.

The method of procedure did not seem to affect the results, since both the tube method and the agar plate method gave substantially the same results.

STANDARDIZATION OF VIBRION SEPTIQUE ANTITOXIN*

By A. S. Schlingman, D.V.M., M.S., Detroit, Mich.

SINCE 1877 when Pasteur first isolated Vibrion septique from animals thought to have died of anthrax, this anaerobe has been isolated from gas infections in horses, cattle, sheep, swine, and man.

During the late war the prevalence of Vibrion septique in war wounds led to the production of an antitoxin for treatment of these conditions. The

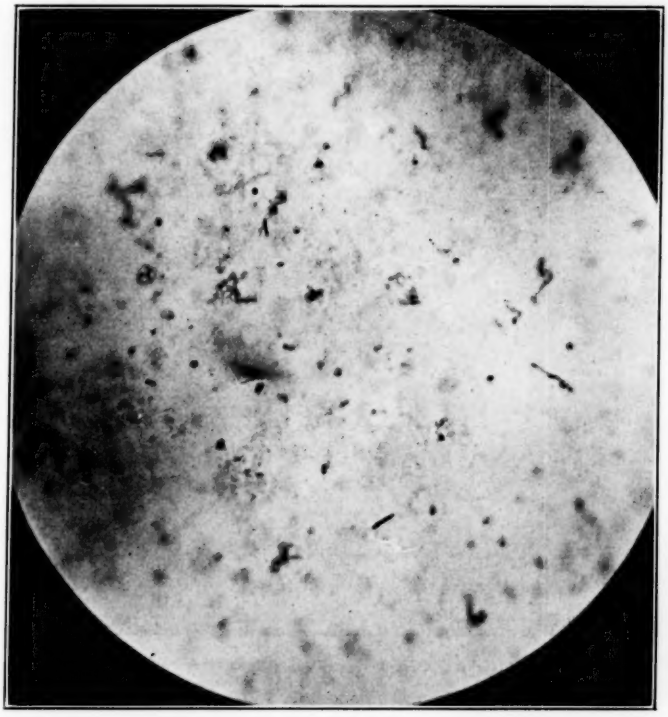

Fig. 1.—Vibrion septique, twenty-four hours Hibler medium culture. (4x eyepiece by 16 mm. objective, 20 inch bellows.)

potency of this antitoxin was measured according to a method devised by Weinberg and Seguin,[1] in which 1-1000 c.c. should neutralize two fatal doses of toxin after thirty minutes' incubation of the mixture at room temperature.

The renewed interest in gas gangrene antitoxin stimulated by an increasing number of cases occurring in civil practice during the past few years, has led to this work to determine a somewhat more accurate method of standardization of Vibrion septique antitoxin.

*From the Research and Biological Laboratories, Parke, Davis and Company.
Received for publication, January 13, 1931.

DESCRIPTION OF VIBRION SEPTIQUE

Vibrion septique is a gram-positive bacillus 3 to 5 microns long by 0.6 to 0.8 microns wide. Spores are oval and are usually median but may be terminal. In Hibler medium, a combination of beef liver bouillon and beef brains, it produces only a slight cloudiness of the liquid after twenty-four hours' incubation at 37° C. There is usually present a rather violent gas production, maximum growth being obtained in eighteen to twenty-four hours. There is no proteolysis of the brain tissue nor is there any odor. In this medium the organisms occur singly, in pairs or in chains (Fig. 1). In old cultures the brain tissue may turn pink and the organisms have a tendency to

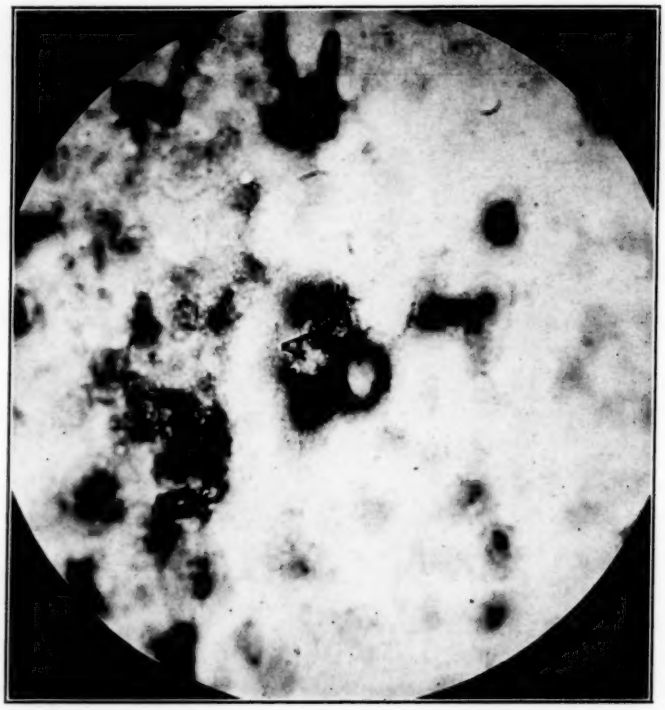

Fig. 2.—Vibrion septique, heart blood of guinea pig. (4x eyepiece by 16 mm. objective, 20 inch bellows.)

settle to the bottom of the tube leaving the liquid clear. In shake cultures in 2 per cent liver agar round, white, woolly colonies are formed, accompanied by early and extensive gas formation which is oftentimes sufficient to shatter the agar. Similar colonies and gas production are seen in a 2 per cent agar medium containing 2 per cent glucose. In litmus milk under oil a soft acid clot is produced in twenty-four to forty-eight hours at 37° C. After eight to ten days' incubation the clot may shrink but it is not digested.

Cultures of Vibrion septique injected subcutaneously into guinea pigs usually cause death in about sixteen hours. The organism usually becomes septicemic before death and as a result no difficulty is experienced in obtaining it from the heart blood when cultures are made immediately after death.

In blood smears made at this time, the bacilli (Fig. 2) appear much as they do in Hibler medium except that they are not so numerous. Chain formation is also sometimes seen. Similar organisms, often in long chains are seen in smears made from the infected muscle (Fig. 3). If impressions are made from the peritoneal surface of the liver, the bacilli may be seen, gram-positive, and occurring singly, in pairs or in long chains (Fig. 4). This chain formation is characteristic and is an aid in differentiating this pathogen from other anaerobes, particularly B. chauvoei.

Gas, edema, and necrosis are produced in the tissues following infection with Vibrion septique (Fig. 5). Macroscopically the affected tissues are hem-

Fig. 3.—Vibrion septique, infected guinea pig muscle. (4x eyepiece by 16 mm. objective, 20 inch bellows.)

orrhagic and the muscle fibers are torn apart by gas. The color varies from pink to a dark shade of red.

While this paper deals primarily with the standardization of Vibrion septique antitoxin, photomicrographs are included with the description of this anaerobe because of some confusion in the literature concerning its identification.

TOXIN PRODUCTION

In culture medium as well as in the animal body, Vibrion septique produces an exotoxin. Bengston[2] used a 0.2 per cent glucose veal broth medium containing 10 per cent of sterile horse serum. A potent toxin can also be produced in horse-meat bouillon containing 0.2 per cent glucose and further

enriched by addition of defibrinated horse blood. In this medium no surface covering with oil is necessary to obtain anaerobiosis providing the Florence flasks used are filled to the neck with medium, the surface exposure thus being reduced to a minimum. Earlier active growth is obtained if the medium is heated and cooled before the addition of horse blood and then seeded while warm.

In this medium, maximum growth usually follows eighteen to twenty-one hours' incubation at 37° C. There is a tendency for the organisms to auto-agglutinate as they become quiescent. Maximum toxin production is usually indicated when the organisms have settled out and the supernatant medium has nearly cleared.

Fig. 4.—Vibrion septique, liver impression, guinea pig. (4x eyepiece by 16mm. objective, 20 inch bellows.)

While a good toxin can usually be produced with little difficulty, it is affected by reaction of filters and the amount of handling necessary during the process of filtration. As a rule best results are obtained when the reaction of the filters, glassware, etc., is P_H 6.0 to P_H 6.2. This is approximately the same as that of the cultures, these reactions having been determined at the end of the incubation periods.

Injected subcutaneously into guinea pigs 2 to 5 c.c. of the toxin rarely cause death but almost invariably produce necrosis at the site of injection. Intravenous injection of an active toxin into rabbits is usually followed by death in from three to ten minutes without any premonitory symptoms.

Robertson[3] has shown that death follows a direct action of the toxin upon the heart. Straub[4] considers this action to resemble that of poisoning with digitalis. Sometimes the action of the toxin may be delayed so that death does not occur until twenty-four or more hours have elapsed, or the animal may show the first disturbance in the heart's action characterized by convulsions or paralysis only to recover.

There seems to be some variation in susceptibility of rabbits to the action of Vibrion septique toxin in that very often those which receive relatively

Fig. 5.—Muscle of guinea pig following infection with Vibrion septique. (4x eyepiece by 16 mm. objective, 20 inch bellows.)

small doses will die after a short time while those receiving larger doses remain unaffected (Table I). Other animals may also show this same idiosyncrasy. Intracardiac injection of the toxin into guinea pigs may be followed by rather erratic results as seen in Table II. Injection into the lymph-sac of

TABLE I

EFFECT OF VIBRION SEPTIQUE TOXIN 094077 INTRAVENOUSLY TO RABBITS

RABBIT	WT. (GM.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	RESULT
1	1560	0.4	0.624	Died—2¾ hours
2	1740	0.5	0.87	Died—40 minutes
3	1540	0.6	0.924	Died—3 hours
4	1260	0.6	0.756	Lived

frogs causes death in a few hours (Table III). White mice show practically the same variation in susceptibility to intravenous injection as do other animals (Table IV.)

The toxin seems to be adversely affected by the addition of trikresol as a preservative (Tables V and VI) even though held at 2-3°C. in sealed bottles. The activity of the toxin is not affected to such a degree by 0.5 per cent phenol (Table VII). On the other hand, there is some very slight deterioration of the toxin when kept in sealed amber bottles at 2-3° C. for fifteen

TABLE II

EFFECT OF INTRACARDIAC INJECTION OF VIBRION SEPTIQUE TOXIN 094561 INTO GUINEA PIGS

PIG	WT. (GM.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	TOXIN 1-10 DIL.	RESULT
1	252	0.35	0.088	0.88	Comatose in one minute. Recovered.
2	248	0.35	0.086	0.86	Not affected.
3	252	0.35	0.088	0.88	Comatose in one minute. Recovered.
4	252	0.35	0.088	0.88	Not affected.
5	232	0.35	0.081	0.81	Not affected.
6	240	0.35	0.084	0.84	Convulsions thirty-five minutes. Recovered.

TABLE III

EFFECT OF INJECTION OF VIBRION SEPTIQUE TOXIN 094118 INTO LYMPH SACS OF FROGS

FROG	WT. (GM.)	TOXIN PER GM. (C.C.)	TOTAL TOXIN (C.C.)	RESULT
1	20	0.01	0.2	Died—2 hours
2	21	0.02	0.42	Died—5 hours
3	19	0.03	0.57	Died—2 hours
4	19	0.04	0.76	Died—2 hours
5	8	0.05	0.4	Died—2 hours

TABLE IV

EFFECT OF INTRAVENOUS INJECTION OF MICE WITH VIBRION SEPTIQUE TOXIN 094062

MOUSE	WT. (GM.)	TOXIN PER 25 GM. (C.C.)	TOTAL TOXIN 1-10 DIL. (C.C.)	RESULT
1	30	0.1	0.12	Not affected
2	30	0.2	0.24	Died— 1 hour
3	26	0.25	0.25	Died— 2 minutes
4	30	0.4	0.48	Died— 1 hour
5	25	0.6	0.6	Died—18 hours
6	30	0.7	0.84	Died— 2 minutes

months (Table VIII) although it was not so great as when a preservative is added and kept under the same conditions of light and temperature.

ANTITOXIN

Horses are rather sensitive to the effects of subcutaneous injection of Vibrion septique toxin, the reactions following its injection being characterized by a rise of temperature (103-106° F.) and formation of swelling at the site of injection. Necrotic areas such as are seen in guinea pigs following

subcutaneous injection of the toxin have never been noted in the horse. This may be due to the fact that the amounts first used are comparatively small and later when larger doses are used, a tolerance for the toxin had been acquired. Horses so treated usually produce a very potent antitoxin.

TABLE V

EFFECT OF INTRAVENOUS INJECTION OF RABBITS WITH VIBRION SEPTIQUE TOXIN 094002 IMMEDIATELY AFTER FILTRATION. (NO PRESERVATIVE ADDED.)

RABBIT	WT. (GM.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	RESULT
1	1720	0.5	0.86	Died— 5 minutes
2	1700	0.6	1.02	Died—10 minutes
3	1720	0.7	1.20	Died—10 minutes
4	1940	0.8	1.55	Died— 5 minutes

TABLE VI

EFFECT OF INTRAVENOUS INJECTION OF RABBITS WITH VIBRION SEPTIQUE TOXIN 094002 TO WHICH 0.4 PER CENT TRIKRESOL WAS ADDED AS PRESERVATIVE. STORED AT 2-3° C.

RABBIT	WT. (GM.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	PRESERVATIVE	AMT.	STORAGE	RESULT
1	1780	0.5	0.89	Trikresol	0.4%	None	Not affected
2	1660	0.6	0.99	Trikresol	0.4%	None	Died—1½ hr.
3	1660	0.7	1.16	Trikresol	0.4%	None	Died—10 min.
4	1720	0.8	1.37	Trikresol	0.4%	None	Died— 9 min.
5	1680	0.6	1.00	Trikresol	0.4%	20 days	Not affected
6	1940	0.7	1.35	Trikresol	0.4%	20 days	Not affected
7	1680	0.8	1.34	Trikresol	0.4%	42 days	Not affected
8	1800	0.9	1.62	Trikresol	0.4%	42 days	Not affected
9	1800	0.5	0.90	None	None	20 days	Died—¼ hr.
10	1800	0.5	0.90	None	None	42 days	Died—18 hr.
11	1720	0.6	1.03	None	None	42 days	Died— 6 min.

TABLE VII

EFFECT OF INTRAVENOUS INJECTION OF VIBRION SEPTIQUE TOXIN 094039 TO WHICH 0.5 PER CENT PHENOL WAS ADDED AS PRESERVATIVE. STORED AT 2-3° C.

RABBIT	WT. (GM.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	PRESERVATIVE	AMT.	STORAGE	RESULT
1	1800	0.6	1.08	None	None	None	Died—3 hr.
2	1530	0.6	0.92	None	None	None	Died—¼ hr.
3	1900	0.6	1.14	Phenol	0.5%	14 days	Died—3½ hr.
4	1820	0.6	1.09	None	None	14 days	Died—¾ hr.
5	2020	0.6	1.21	Phenol	0.5%	30 days	Died—52 hr.
6	2240	0.6	1.34	None	None	30 days	Died—20 hr.

TABLE VIII

EFFECT OF INTRAVENOUS INJECTION OF RABBITS WITH VIBRION SEPTIQUE TOXIN 094077 HELD AT 2-3° C. FOR FIFTEEN MONTHS

RABBIT	WT. (GM.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	STORAGE	RESULT
1	1560	0.4	0.624	None	Died—2¾ hr.
2	1700	0.4	0.68	15 mo.	Died—12 hr.

STANDARDIZATION OF ANTITOXIN

On account of the variation in susceptibility of most animals to the action of the toxin and the resultant difficulty in measuring accurately the potency of the antitoxin, experiments were undertaken to devise a method of standardization of the antitoxin in which these variabilities might be reduced to a minimum.

While the method here described is not basically new, being somewhat similar to those used for determination of potency of diphtheria, tetanus and perfringens antitoxins, yet certain changes have been made in order to meet the requirements of this particular case.

On account of the ease with which intravenous injections can be made into the ear vein, rabbits are used as test animals.

In the determination of the potency of Vibrion septique antitoxin the following standards are used:

1. *M.L.D. of Toxin.*—The M.L.D. of toxin is the smallest amount per kilogram of rabbit which, when injected intravenously, will cause death within twenty-four hours. (Table IX.)

TABLE IX

DETERMINATION OF M.L.D. OF VIBRION SEPTIQUE TOXIN 094561

RABBIT	WT. (GM.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	RESULT
1	1550	0.32	0.49	Lived
2	1650	0.33	0.54	Lived
3	1800	0.34	0.61	Lived
4	2000	0.35	0.70	Died less than 16 hr.
5	1860	0.40	0.74	Died less than 16 hr.
		M.L.D. = 0.35 c.c. per kg.		

The rabbits are weighed accurately and the amount of toxin per kilogram is computed to two decimal places. Twenty-four hours is used as the time limit since it has been noted that often there may be an initial reaction shortly after injection followed by recovery only to be followed later by death.

2. *Test Dose of Toxin.*—The test dose of toxin is the smallest quantity of toxin per kilogram of rabbit, injected intravenously, which will cause death

TABLE X

DETERMINATION OF TEST DOSE OF TOXIN 094561. STANDARD ANTITOXIN 094590 DILUTED SO THAT 0.1 C.C. CONTAINED 1/10 UNIT

RABBIT	WT. (GM.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	ANTI-TOXIN PER KG. (C.C.)	TOTAL ANTI-TOXIN (C.C.)	AMT. 1-10 DIL.	RESULT
1	2150	3.5	7.52	0.1	0.215	2.15	Lived
2	2300	3.67	8.45	0.1	0.23	2.3	Died 2 hr.
3	1800	3.85	6.93	0.1	0.16	1.8	Died 2 hr.
4	2100	4.02	8.44	0.1	0.21	2.1	Died 1 hr.
5	1925	0.35	0.67	Control			Died 12 hr.
			Test Dose = 3.67 c.c. per kg.				

in twenty-four hours, when mixed with $\frac{1}{10}$ unit of standard antitoxin. The mixture of toxin and antitoxin is allowed to stand thirty minutes at 37° C. before injection (Table X). A standard antitoxin, carefully tested, is held at 2-3° C. and used for comparison. Experiments are under way to determine the effect of desiccation on the antitoxin with the hope that an even more stable standard may be obtained.

3. *Antitoxic Unit.*—A unit of Vibrion septique antitoxin is that quantity which will neutralize 100 M.L.D. of Vibrion septique toxin per kilogram of body weight of rabbit. Rabbits weighing 1.5 to 2 kilograms are preferred.

4. *Potency Test.*—Potency of an unknown antitoxin is determined by injecting a test dose of toxin and variable amounts of the unknown antitoxin intravenously into rabbits. The smallest quantity of antitoxin per kilogram which will protect the rabbit against a test dose of toxin, contains $\frac{1}{10}$ unit (Table XI).

TABLE XI

STANDARDIZATION OF VIBRION SEPTIQUE ANTITOXIN 010164.
TEST DOSE OF TOXIN 094561 = 3.67 C.C.

RABBIT	WT. (KG.)	TOXIN PER KG. (C.C.)	TOTAL TOXIN (C.C.)	ANTI-TOXIN PER KG. (C.C.)	TOTAL ANTI-TOXIN (C.C.)	ANTI-TOXIN 1-100 DIL.	RESULT
1	1950	3.67	7.16	0.0025	0.0048	0.48	Died 10 min.
2	1400	3.67	5.14	0.003	0.0042	0.42	Died 10 min.
3	2000	3.67	7.35	0.0035	0.007	0.7	Lived
4	1700	3.67	6.25	0.00375	0.0063	0.63	Lived
5	1350	3.67	4.96	0.1 (Std.)	0.135	1.35 (1-10)	Died 12 hr.
6	1700	3.67	6.25	Control			Died 12 hr.
			0.0035 c.c. contains 1/10 unit.				
			1 c.c. contains 28.5 units.				

SUMMARY

A description of the growth of Vibrion septique in Hibler medium, 2 per cent glucose agar, 2 per cent liver agar and in horse meat bouillon containing 0.2 per cent glucose and defibrinated horse blood, is given.

The variability in susceptibility of various animals is shown by the effect of injection of Vibrion septique toxin into rabbits, guinea pigs, mice, and frogs.

Horses are rather sensitive to injections of Vibrion septique toxin but with care in hyperimmunization a potent antitoxin can be produced.

In order to overcome some of the difficulties found in standardizing Vibrion septique antitoxin due to the variation in susceptibility of test animals to the toxin, a new method of measuring the potency of this antitoxin is suggested.

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A COMPARISON OF METHODS FOR DETERMINING THE SIZE OF THE ERYTHROCYTE*

By Russell L. Haden, M.D., Cleveland, Ohio

BECAUSE of the clinical value of the determination of the size of the erythrocyte, it seems well to offer a description of a comparative study of the methods that have been used. In 1674 Leeuwenhoek[1] made the first measurements by comparing the diameter of the red cell with that of a grain of sand, which was his unit of measurement for microscopic objects. He considered the diameter of 100 sand grains as equal to one inch and found the diameter of the erythrocyte to be one-thirtieth of that of a grain of sand, or 8.5 microns. Later Jurin[2] measured the diameter of a single red cell by comparing it with the diameter of silver wire. This observer first wound the finest silver wire tightly about a pin; he then measured the length of the pin, counted the number of turns of wire, and thus determined the diameter of the wire to be one four hundred and eighty-fifth of an inch. The wire was then cut into very small pieces, mixed with the blood and observed under the microscope. By this method Jurin found that four "globules" of human blood would generally cover the breadth of a wire so that the diameter of the single erythrocyte was one-one thousand nine hundred and fortieth of an inch, or 13.1 microns. Leeuwenhoek confirmed this measurement by using a piece of the same wire which had been sent to him by Jurin.[2]

With the development of the micrometer eyepiece, workers used this method for measuring the diameter of the red cells of man, securing approximately correct results. Other workers have projected the cells with a camera lucida at a known magnification and have measured them directly with a millimeter rule. This method is used by Price-Jones[3] who is largely responsible for the renewed interest which is being shown in the measurement and clinical application of the diameter of the red cell. A filar micrometer employing a moving hair is more satisfactory for cell measurements than is the fixed micrometer eyepiece, and this has been used in the present study. Jörgensen and Warburg[4] have reviewed the micrometric findings of various observers.

Recently diffraction methods have again been used for measuring cell diameters. Thomas Young[5] (1813) was the first to show that the principle of diffraction could be used practically in the measurement of small objects, and he constructed an instrument with which he measured red blood cells and other objects. This principle was again applied by Pijper[6] and has been utilized practically in the eriometer of Emmons[7] and the halometer of Eve.[8] By the direct micrometric measurement of a certain number of cells the mean

*From the Cleveland Clinic.
Received for publication, January 30, 1931.

diameter is calculated and from the data thus obtained a distribution curve is plotted, as suggested by Price-Jones. Only the mean diameter is determined by the various diffraction methods.

Determinations of the volume of the red cell were first made by Welcker in 1864.[9] With the aid of the microscope, Welcker made plaster models of blood corpuscles at a definite magnification, weighed them and calculated the average volume as 72 cubic microns. The discovery of the hematocrit offered a much more accurate method of determining the cell volume and this method has been used by most workers. The cell volume can also be determined from the change in concentration of different plasma constituents when whole blood is diluted to exactly twice its volume with isotonic saline solution. Bleibtreu,[10] who introduced this method, determined the plasma protein. Emmons[11] determined the blood sugar. The refractive index[12] of plasma and the viscosity[13] can be similarly determined. Stewart[14] determined the cell volume by electrical conductivity measurements and also by a colorimetric method.

Numerous attempts have been made to calculate the cell volume from measurements of the diameter and thickness of the cells. It is difficult to measure the thickness of the cell and the calculation of the volume is not simple on account of the shape of the erythrocyte. Jörgensen and Warburg[4] have shown that the mean diameter of the cells in a given specimen of blood can be calculated if the cell volume relative to the normal volume is known. If the volume index (VI) is taken as the index of the average relative cell volume then:

$$\text{Calculated mean diameter} = \text{normal mean diameter} \times \sqrt[3]{\text{VI}}$$

In the experiments reported in this paper, the cell volume in cubic microns has been determined in each specimen of blood by the hematocrit method previously described.[15] A film was made on cover slips by blood drawn from the finger tip at the same time that the blood was withdrawn from a vein for the determination of the cell volume and the film was stained by Wright's method. In each film preparation 100 cells were measured by means of a filar micrometer, which had been previously calibrated with a stage micrometer. The average normal diameter was found to be 7.7 microns. From the cell volume determination the volume index was calculated. The calculated mean diameter was figured from the volume index and the average normal diameter by Jörgensen and Warburg's formula. The mean diameter was also read directly on each blood film by means of an Emmons eriometer.

The results of this study of 10 normal individuals, 50 patients with pernicious anemia and 50 patients with secondary anemia of different types are shown in Tables I to III.

In 10 normal men and women (Table I), the average corpuscular volume was 92 cubic microns with a variation of from 87 to 97 cubic microns. The volume index varied from 0.95 to 1.05 with an average of exactly 1.00. The cell diameter showed very little variation (7.3-8.0) with a mean of 7.7. This was taken as the normal mean diameter in calculating the diameter from the cell volume by Jörgensen and Warburg's formula. Since the volume

TABLE I

FINDINGS IN NORMAL PERSONS

NO.	RED BLOOD CELLS	HEMATOCRIT READING PER 100	AVERAGE CORPUSCULAR VOLUME	VOLUME INDEX	CALCULATED MEAN DIAMETER	MEASUREMENT (DIAMETER) WITH			
						FILAR MICROMETER			ERIOMETER
						MEAN	MAXIMUM	MINIMUM	
	Millions	c.c.	Cubic Microns		Microns	Microns	Microns	Microns	Microns
1	5.25	48	91	0.99	7.7	7.5	9.6	5.6	7.8
2	5.12	47	92	1.00	7.7	7.8	9.6	5.4	7.7
3	4.51	39	87	0.95	7.6	7.7	9.2	5.0	7.6
4	4.73	42	89	0.97	7.7	7.3	9.4	5.0	7.7
5	4.95	45	91	0.99	7.7	7.4	9.2	6.0	7.7
6	4.99	47	94	1.02	7.7	7.6	9.2	6.0	7.8
7	4.03	38	95	1.03	7.8	7.6	9.4	5.4	7.9
8	4.00	37	93	1.01	7.7	7.9	9.2	6.0	7.7
9	4.66	45	97	1.05	7.8	8.0	9.4	6.6	8.0
10	4.62	42	92	1.00	7.7	8.0	9.6	6.6	8.0
Average	4.69	43	92	1.00	7.7	7.7	9.4	5.8	7.8

index is 1.00 the calculated mean diameter is the same (7.7), the calculated mean diameter varying only from 7.6 to 7.8 microns.

The maximum and minimum limits of cell diameter are also quite constant. The largest cell was 9.6 microns and the smallest 5.4 microns, the average limits being 9.4-5.8. Measurements with the eriometer check closely with those made with the filar micrometer. The average diameter is 7.8 microns with a variation of 7.7 to 8.0 microns.

The findings in 50 cases of pernicious anemia are shown in Table II. The average corpuscular volume is 132 cubic microns and the volume index 1.44. The variation in corpuscular volume is very great (106 to 179 cubic microns), corresponding to the variation in the volume index of from 1.15 to 1.95. The average cell diameter as determined with the filar micrometer is 8.88 microns with a variation of from 11.4 to 6.2 microns. The largest cell seen measured 13.0 microns and the smallest 5.2 microns. The calculated mean diameter is 8.76 as compared with a measured diameter of 8.88 microns. The diameter was determined by means of the eriometer in only 33 of the 50 cases. In this group of 33 cases the average cell diameter, when measured with the eriometer, was 8.7 microns, as compared with a diameter of 9.0 microns, when measured with the filar micrometer.

The findings in 50 cases of secondary anemia are shown in Table III. The average corpuscular volume is 88 cubic microns with a variation of from 63 to 109 cubic microns. In only three of the 50 cases is the corpuscular volume greater than the upper limit of normal (1.05). The volume index varied from 0.63 to 1.19 with a mean of 0.96. The calculated mean diameter was 7.55 microns as compared with a diameter of 7.57 microns determined by means of the filar micrometer. Large cells were often found, the largest measuring 12.2 microns in diameter; the smallest cell seen measured 4.0 microns in diameter. Measurements were made by means of the eriometer in 41 of the 50 cases of secondary anemia. The average diameter is 7.6 microns as compared with 7.6 microns when measured with the filar micrometer.

DISCUSSION

The comparative value of the volume index and the cell diameter measurements has been discussed in a previous paper.[15] The measurement of total cell volume is a much more sensitive indicator of change in size than is the cell diameter. In the series of cases of pernicious anemia reported in this paper there is an increase in volume of 40 cubic microns or 44 per cent. The average increase in cell diameter is 1.18 microns or 15 per cent. The volume varies with the cube of the diameter of the cell, hence the marked increase in the volume as compared with the diameter.

In calculating the mean diameter in specimens of blood showing marked anisocytosis a correction should be subtracted from the mean diameter. This correction is so small, however, that it may be disregarded for practical purposes.

In the series of cases reported here the results obtained by means of the eriometer check closely with the measurements made with the filar micrometer. The average cell diameter is much more valuable in making a diagnosis

TABLE II

FINDINGS IN PERNICIOUS ANEMIA

NO.	RED BLOOD CELLS	HEMATOCRIT READING PER 100	AVERAGE CORPUSCULAR VOLUME	VOLUME INDEX	COLOR INDEX	SATURATION INDEX	CALCULATED MEAN DIAMETER	MEASUREMENTS (DIAMETER) WITH			
								FILAR MICROMETER			ERIOMETER
								MEAN	MAXIMUM	MINIMUM	
	Millions	c.c.	Cubic Microns				Microns	Microns	Microns	Microns	Microns
1	0.45	6.9	152	1.66	1.33	0.80	9.1	8.9	13.0	7.0	----
2	0.60	6.9	115	1.25	1.25	1.00	8.3	8.5	11.6	6.6	8.5
3	0.66	9.8	149	1.62	1.62	1.00	9.1	9.6	12.2	5.0	8.9
4	0.69	9.8	143	1.56	1.43	0.90	8.9	8.8	11.2	5.8	----
5	0.86	11	134	1.46	1.34	0.92	8.8	9.2	11.6	5.6	9.0
6	0.87	10	120	1.30	1.35	1.04	8.4	8.7	12.0	5.2	----
7	0.97	13	143	1.56	1.51	0.97	8.6	8.7	11.6	5.3	8.4
8	0.98	12	132	1.43	1.43	1.00	8.7	8.7	11.4	5.8	----
9	0.99	12	129	1.40	1.40	1.00	8.6	8.7	12.2	6.6	8.5
10	1.00	11	115	1.25	1.05	0.84	8.3	9.1	11.6	7.2	----
11	1.02	12	124	1.40	1.24	0.89	8.5	8.5	11.4	5.4	8.5
12	1.05	19	179	1.95	1.52	0.75	10.1	9.7	11.8	7.2	9.0
13	1.05	17	166	1.81	1.81	1.00	9.4	9.5	11.6	5.6	9.0
14	1.10	18	167	1.82	1.91	1.05	9.5	9.0	12.4	6.4	9.1
15	1.13	15	134	1.46	1.46	1.00	8.8	8.4	10.8	6.2	----
16	1.20	15	127	1.38	1.25	0.91	8.6	8.5	11.6	6.6	8.1
17	1.24	19	154	1.68	1.32	0.79	9.2	9.2	11.2	6.0	----
18	1.26	19	154	1.68	1.68	1.00	9.0	9.3	11.2	7.4	9.5
19	1.35	18	145	1.58	1.04	0.70	8.8	9.5	12.4	7.2	8.5
20	1.48	20	140	1.53	1.53	1.00	8.9	8.7	11.4	5.2	----
21	1.50	25	172	1.87	1.60	0.86	9.2	9.0	12.0	6.8	9.0
22	1.55	22	140	1.52	1.48	0.96	8.9	9.0	12.0	5.0	----
23	1.58	27	133	1.45	1.59	0.96	8.7	8.8	11.2	6.6	8.8
24	1.65	23	145	1.58	1.60	1.02	9.0	9.5	12.4	7.4	8.8
25	1.66	20	124	1.33	1.21	0.90	8.5	9.0	12.4	5.2	8.7

TABLE II—CONT'D

NO.	RED BLOOD CELLS	HEMATOCRIT READING PER 100	AVERAGE CORPUSCULAR VOLUME	VOLUME INDEX	COLOR INDEX	SATURATION INDEX	CALCULATED MEAN DIAMETER	MEASUREMENTS (DIAMETER) WITH FILAR MICROMETER			ERIOMETER
								MEAN	MAXIMUM	MINIMUM	
	Millions	c.c.	Cubic Microns				Microns	Microns	Microns	Microns	Microns
26	1.70	22	130	1.41	1.30	9.92	8.7	9.8	12.2	5.2	9.2
27	1.78	23	132	1.44	1.30	0.90	8.7	9.1	11.8	7.2	8.4
28	1.79	21	121	1.31	1.14	0.87	8.4	8.9	11.6	6.6	----
29	1.85	29	156	1.70	1.62	0.95	9.2	8.9	11.6	6.6	9.0
30	1.96	25	131	1.43	1.46	1.02	8.7	8.1	11.0	6.2	----
31	2.02	21	106	1.15	1.15	1.00	8.1	8.6	10.4	6.8	8.2
32	2.04	29	146	1.59	1.51	0.95	9.0	8.5	9.8	7.0	8.7
33	2.08	31	155	1.68	1.53	0.91	9.2	9.0	11.4	7.6	8.7
34	2.25	28	129	1.40	1.15	0.80	8.6	8.8	11.4	7.2	8.8
35	2.42	29	120	1.31	1.29	0.95	8.4	9.1	11.2	7.2	9.3
36	2.42	32	136	1.48	1.42	0.96	8.7	9.1	11.2	6.8	9.2
37	2.50	34	138	1.50	1.38	0.92	8.8	8.6	11.8	6.6	9.0
38	2.60	33	129	1.40	1.38	0.99	8.6	9.3	11.0	7.0	9.3
39	2.60	29	117	1.27	1.22	0.96	8.4	8.8	10.8	5.2	8.8
40	2.64	34	130	1.41	1.38	0.98	8.7	8.6	11.0	6.8	----
41	2.65	39	150	1.63	1.54	0.94	9.1	8.6	10.0	6.0	----
42	2.79	31	113	1.23	1.16	0.94	8.3	8.0	10.0	5.2	----
43	2.79	41	148	1.61	1.44	0.90	9.0	8.8	12.2	6.8	8.8
44	2.80	34	122	1.34	1.30	0.97	8.5	9.0	11.2	6.6	----
45	2.85	32	113	1.23	1.23	1.00	8.3	8.7	10.6	7.4	8.1
46	3.10	34	111	1.21	1.23	1.02	8.7	8.6	11.4	5.6	----
47	3.13	33	107	1.16	0.94	0.81	8.1	8.1	10.2	6.6	8.4
48	3.16	40	134	1.46	1.30	0.90	8.8	9.2	10.6	7.2	8.6
49	3.22	41	130	1.41	1.41	1.00	8.7	8.7	10.4	7.0	7.7
50	3.60	43	119	1.29	1.29	1.00	8.4	8.5	10.6	7.0	----
Average	1.81	24	132	1.44	1.35	0.94	8.76	8.88	11.39	6.25	----

Table III

Findings in Secondary Anemia

NO.	RED BLOOD CELLS	HEMATOCRIT READING PER 100	AVERAGE CORPUSCULAR VOLUME	VOLUME INDEX	COLOR INDEX	SATURATION INDEX	CALCULATED MEAN DIAMETER	MEASUREMENTS (DIAMETER)				DIAGNOSIS
								WITH FILAR MICROMETER			ERIOMETER	
								MEAN	MAXIMUM	MINIMUM		
	Millions	c.c.	Cubic Microns				Microns	Microns	Microns	Microns	Microns	
1	1.14	10	92	1.00	0.87	0.87	7.7	8.5	10.8	6.0	8.3	Essential thrombopenia
2	1.38	12	91	0.99	0.90	0.93	7.7	7.8	9.2	6.2	--	Essential thrombopenia
3	1.50	15	105	1.14	1.10	0.97	8.1	7.6	12.2	5.0	7.5	Acute hemolytic jaundice
4	1.52	14	92	1.00	1.03	1.03	7.7	7.7	10.0	6.0	8.1	Osteitis fibrosa
5	1.53	14	97	1.05	0.69	0.69	7.8	7.3	10.2	5.2	--	Hemorrhoids
6	1.61	15	92	1.00	0.75	0.70	7.7	7.7	10.2	6.2	7.7	Malnutrition
7	1.80	14	79	0.86	0.56	0.65	7.3	7.3	9.8	5.0	--	Anemia of pregnancy
8	1.84	15	82	0.89	0.80	0.94	7.4	7.1	9.8	5.0	7.1	Essential thrombopenia
9	1.85	20	109	1.19	0.81	0.67	8.2	7.6	9.6	5.8	7.8	Carcinoma of stomach
10	1.98	18	92	1.00	0.96	0.96	7.7	7.8	9.8	5.6	7.5	Menorrhagia
11	1.98	15	78	0.85	0.85	0.67	7.3	7.5	10.0	5.0	--	Essential thrombopenia
12	2.04	18	90	0.98	0.70	0.72	7.7	7.6	10.4	5.4	--	Achylia
13	2.04	19	94	1.02	0.73	0.72	7.7	7.9	10.8	5.8	7.6	Menorrhagia
14	2.47	17	75	0.81	0.71	0.90	7.2	7.7	10.0	6.2	7.6	Carcinoma of stomach
15	2.50	24	99	1.08	0.98	0.91	7.9	7.9	9.4	5.4	7.9	Hemophilia
16	2.52	23	92	1.00	0.74	0.74	7.7	7.6	9.2	5.8	--	Achlorhydria
17	2.56	22	86	0.94	0.82	0.87	7.6	8.0	9.8	5.4	8.2	Cystitis and pyelitis
18	2.62	23	89	0.97	0.89	0.88	7.7	7.7	9.2	5.8	7.9	Essential thrombopenia
19	2.75	23	83	0.90	0.76	0.84	7.4	7.3	9.0	5.8	7.5	Thrombophlebitis
20	2.63	24	93	1.01	0.95	0.93	7.9	8.2	10.4	6.4	8.0	Monocytic leucemia
21	2.77	19	70	0.76	0.64	0.79	7.0	7.1	10.2	5.4	7.2	Achylia
22	2.83	28	100	1.09	0.87	0.81	8.0	8.3	10.4	6.2	--	Dietary deficiency
23	2.87	19	66	0.72	0.60	0.83	6.9	6.8	8.4	4.8	7.2	Carcinoma of sigmoid
24	3.05	28	93	1.01	0.91	0.90	7.7	7.9	9.6	6.4	7.7	Hemophilia
25	3.07	24	83	0.90	0.60	0.66	7.4	6.9	8.6	5.8	7.5	Hemorrhoids
26	3.10	28	91	0.99	0.83	0.84	7.7	8.2	10.0	6.4	--	No diagnosis

TABLE III—CONT'D

NO.	RED BLOOD CELLS	HEMATOCRIT READING PER 100	AVERAGE CORPUSCULAR VOLUME	VOLUME INDEX	COLOR INDEX	SATURATION INDEX	CALCULATED MEAN DIAMETER	MEASUREMENTS (DIAMETER) WITH FILAR MICROMETER			ERIOMETER	DIAGNOSIS
								MEAN	MAXIMUM	MINIMUM		
	Millions	c.c.	Cubic Microns				Microns	Microns	Microns	Microns	Microns	
27	3.12	23	74	0.80	0.80	1.00	7.2	7.0	9.2	5.0	7.4	Menorrhagia
28	3.20	29	93	1.01	0.81	0.77	7.7	7.5	8.8	5.8	7.4	Menorrhagia
29	3.20	27	86	0.94	0.80	0.85	7.6	7.6	9.6	5.0	8.2	Achlorhydria
30	3.25	30	97	1.05	1.00	0.96	7.8	8.3	11.6	4.8	8.3	Carcinoma of pancreas
31	3.34	32	97	1.05	0.86	0.83	7.8	8.1	10.6	6.0	8.4	No diagnosis
32	3.40	32	95	1.03	0.96	0.93	7.5	7.9	9.8	6.6	8.6	Myxedema
33	3.40	24	74	0.80	0.66	0.83	7.1	6.8	8.4	4.8	7.5	Uterine hemorrhage
34	3.45	28	89	0.97	0.87	0.90	7.6	7.3	9.4	5.8	7.6	Chronic cholecystitis
35	3.50	28	87	0.95	0.93	0.97	7.6	7.1	9.0	5.6	7.7	Menorrhagia
36	3.71	29	81	0.88	0.57	0.60	7.4	7.6	10.6	5.4	8.2	Ochronosis
37	3.76	34	92	1.00	0.81	0.81	7.7	7.4	9.0	5.8	7.7	Achlorhydria
38	3.76	34	92	1.00	1.00	1.00	7.7	7.6	9.2	6.2	7.7	Hemophilia
39	3.79	28	76	0.83	0.62	0.75	7.2	6.9	8.4	5.2	7.7	Achylia
40	3.82	33	88	0.96	0.96	1.00	7.6	8.0	9.6	6.4	7.9	No diagnosis
41	3.84	36	93	1.01	0.94	0.81	7.7	7.9	9.8	5.4	7.7	Achlorhydria
42	3.85	34	87	0.95	0.89	0.93	7.7	8.3	9.2	7.0		Cirrhosis of liver
43	3.92	37	95	1.03	0.90	0.89	7.8	7.6	9.6	5.0	7.7	Chronic hemolytic jaundice
44	3.92	34	87	0.95	0.60	0.69	7.6	7.5	9.6	5.8	8.0	No diagnosis
45	4.02	31	79	0.86	0.56	0.65	7.3	7.2	8.8	5.2	7.4	Hemorrhoids
46	4.18	34	82	0.89	0.52	0.74	7.4	6.7	8.4	5.2	7.2	Achlorhydria
47	4.25	35	83	0.90	0.79	0.92	7.5	7.6	9.2	5.4	8.2	Menorrhagia
48	4.30	24	58	0.63	0.37	0.59	6.6	6.5	8.0	4.0	7.5	Chlorosis
49	4.50	28	68	0.74	0.60	0.81	7.0	7.4	9.2	6.0	7.4	Chronic cholecystitis
50	4.59	35	77	0.84	0.73	0.87	7.3	7.8	9.2	5.6	8.1	Hemorrhoids
Average	2.89	25	88	0.96	0.79	0.83	7.55	7.57	9.63	5.62		

than is the distribution curve, and it is doubtful if the added information obtained by the distribution curve is worth the time required to make measurements of individual cells. The calculated mean diameter checks closely with the filar micrometer and eriometer measurements.

SUMMARY

1. The results of simultaneous determinations of the corpuscular volume, the calculated mean diameter and measurements of the diameter by means of the filar micrometer and the eriometer, are reported.

2. It was found that the findings from the three methods of determining the corpuscular diameter checked closely.

3. The additional information obtained from the actual measurement of the diameter of a cell probably does not justify the time required to make this measurement.

4. The Emmons eriometer is a satisfactory instrument for the approximate determination of the average cell diameter.

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LABORATORY METHODS

THE IMPORTANCE OF THE TIME OF SEARCH IN EXAMINING STAINED PREPARATIONS FOR RARE TUBERCLE BACILLI*

By Joseph E. Pottenger, A.B., M.D., Monrovia, California

NO DOUBT all workers have a general understanding of the importance of the time of search in making a microscopic diagnosis when tubercle bacilli are rare. However, the relation of the time of search to the results obtained in practice appears not to have been determined.

In comparing the relative efficiency of various technics for the detection of rare bacilli, as reported in the literature, often the time of search is not mentioned at all; in other instances, workers admit that they do not employ a uniform time standard.

Ellerman and Erlandsen[1] who made a most painstaking research on the comparative efficiency of six different methods, based their conclusions entirely upon the relative number of bacilli per field in preparations made from unit volume of centrifuged residue spread uniformly over an area of standard size, that is, upon the relative degree of concentration of bacilli attained by the various methods. Berry and Smeaton[2] in comparing the efficiency of the Ellerman and Erlandsen with the Kinyoun modification of the ligroin-antiformin method suggested that "a longer search may account for some of the differences in results" obtained. Kinyoun[3] writing of the time saved by

Table I

Records of Rare Bacilli Found in Various Sputa During a Fifteen-Minute Search

	Group I			Group II			Group III		
Min.	6517	9018	27315	6371	9077	27345	10881	27318	27356
1	0	0	1	1-2-1-1-2	1	0	0	0	1
2	4	0	1-1-1-1	2	0	1	1	1	0
3	0	0	2	1	0	1-1-1	0	0	0
4	3	1	0	1-1	0	1	1-1	0	0
5	0	5	1	1	1	0	1	0	1-1
6	0	0	0	0	0	0	0	1	0
7	0	0	0	7	1	4	0	0	0
8	0	1	4	1-2	1	0	0	0	0
9	0	1	0	2	0	0	2	0	0
10	0	1	0	0	0	0	0	1	1-1
11	0	0	0	1	1	0	1	0	0
12	0	0	0	0	0	0	0	0	0
13	0	0	0	0	5	1	1	0	0
14	0	0	0	1	1	0	1-1	0	1
15	0	0	0	0	1-1	2	0	0	1

A horizontal line over the findings indicates their occurrence in the same field.

*From the Laboratory of The Pottenger Sanatorium, Monrovia, California.
Received for publication, December 27, 1930.

his methods says, "If after a careful search of one minute you do not find bacilli, it is useless to search longer."

Some years ago, while studying the distribution[4] of bacilli in stained preparations, I established fifteen minutes as my time standard, in case bacilli were rare or negative. If rare, that is, one or less per minute's search, a detailed record was made indicating: (a) the occurrence of the bacilli in groups or as free cells; (b) the estimated number of bacilli in each group; (c) the minute during which the findings were made, and (d) the fact that two or more findings occurred in the same field. Table I presents a few of these records.

The difficulty in finding bacilli is conditioned by their rarity and their poor distribution in the preparation to be examined. Many preparations, as is well known, show plenty of bacilli over a period of time; but the bacilli are so poorly distributed that many fields yield nothing, while in other fields bacilli are plentiful. Consequently, as time is the basis of this study, I assume rare bacilli to be of such number and distribution that at least a one-minute period of the total fifteen minutes, fails to yield positive results. Only such records as conform to this definition are included in this study.

TABLE II

TABULATION OF RECORDS BY GROUPS

NUMBER OF SPECIMENS		TOTALS			AVERAGES		
		GROUP I	GROUP II	GROUP III	GROUP I	GROUP II	GROUP III
		110	200	110	–	–	–
Findings	Free Bacilli	1098	962	457	10.0	4.8	4.2
	Groups	342	503	23	3.1	2.5	0.2
	Free Bacilli Plus Groups	1440	1465	480	13.1	7.3	4.4
	Total Bacilli	2141	2657	508	19.5	13.3	4.6
Total Minutes Positive		693	1004	365	6.3	5.0	3.3

During the accumulation of these records various technics were employed, securing different degrees of distribution and concentration of bacilli. I have divided the records into three groups according to the degree of distribution attained. Group I includes direct smears from fresh material and from material which has fermented in the oven, and a few preparations each by the antiformin and the Ellerman and Erlandsen methods. Group II includes all preparations in which the shaking machine was used alone, and Group III includes those in which both shaking machine and enrichment with a hydrocarbon were used. Most of these specimens were enriched with gasoline, although xylol, ligroin, and benzol were employed in a few preparations with similar results. The preparations were stained with carbolfuchsin, decolorized with 1 to 2 per cent nitric acid, followed by 70 per cent alcohol and counterstained with 1 per cent picric acid or less frequently with Loeffler's methylene-blue. The tabulation of these records by groups is given in Table II.

Table III gives the number of positive diagnoses for each time period (one minute, two minutes, etc., up to fifteen minutes) for each group. Below

TABLE III

NUMBER OF FIRST FINDINGS AND PERCENTAGES WITHIN ANY TIME PERIOD FROM 1 UP TO 15 MINUTES

MINUTES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
GROUP I															
First findings	44	60	75	83	87	90	93	98	102	105	105	108	109	109	110
Percentages	40	54.5	68.2	75.5	79.1	81.8	84.5	89.1	92.7	95.5	95.5	98.2	99.1	99.1	100
GROUP II															
First findings	49	90	115	127	144	147	160	169	175	178	182	187	192	198	200
Percentages	24.5	45	57.5	63.5	72	73.5	80	84.5	87.5	89	91	93.5	96	99	100
GROUP III															
First findings	21	42	56	65	72	83	87	91	96	101	106	107	108	109	110
Percentages	19.1	38.2	50.9	59.1	65.5	75.5	79.1	82.7	87.3	91.8	96.4	97.3	98.2	99.1	100

these figures are given the percentage diagnoses for each period. These percentage figures are plotted on the chart to form the experience curves.

Checking experience by theory, certain mathematical principles are to be considered. The problem presents a variation in the number of positive minute periods; they may vary from one, two, three, etc., up to fourteen. Each of these varying number of minute periods gives a distinct series of arrangements. To determine the number of possible arrangements for each series the general formula for combinations is applicable

$n\ C\ r = \frac{n!}{r!\ (n-r)!}$ where n equals the total number of minute periods (15) and r represents in succession for each series one, two, three, etc., up to fourteen, positive minute periods. It is necessary to distribute the total number of combinations through the series, giving to each minute period its appropriate value. The same formula may be used to determine these values; but the arrangements are somewhat restricted by the nature of the problem, for instance n equals 15 for the first minute, $n-1$ equals 14 for the second minute, etc., decreasing by 1 for each succeeding minute. Since the first minute must be positive for all possible succeeding arrangements, there are left $r-1$ minute periods to be arranged in $n-1$ places. Similarly for the second minute $r-1$ minute periods are to be arranged in $n-2$ places, etc. A more direct way of applying the principle is by the expansion of Pascal's Triangle to the fourteenth series. This is set out in Table IV.

TABLE IV

INCREASE FOR EACH SUCCEEDING MINUTE FOR EACH VALUE OF r

MIN.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	NCR
$r=1$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
$r=2$	14	13	12	11	10	9	8	7	6	5	4	3	2	1		105
$r=3$	91	78	66	55	45	36	28	21	15	10	6	3	1			455
$r=4$	364	286	220	165	120	84	56	35	20	10	4	1				1365
$r=5$	1001	715	495	330	210	126	70	35	15	5	1					3003
$r=6$	2002	1287	792	462	252	126	56	21	6	1						5005
$r=7$	3003	1716	924	462	210	84	28	7	1							6435
$r=8$	3432	1716	792	330	120	36	8	1								6435
$r=9$	3003	1287	495	165	45	9	1									5005
$r=10$	2002	715	220	55	10	1										3003
$r=11$	1001	286	66	11	1											1365
$r=12$	364	78	12	1												455
$r=13$	91	13	1													105
$r=14$	14	1														15

In the first series, $r = 1$, there is but one way by which the first or any other minute period may give the first positive finding; hence, the value of each minute period is 1. This row of 1's, forming series $r = 1$, forms one side of Pascal's Triangle (Table IV). When $r = 2$ there is only one way in which the fourteenth minute may give the first positive finding. When $r = 3$, there is but one way in which the thirteenth minute may give the first positive finding, etc., up to $r = 14$. The arrangement of these 1's for each series in succession from above downward forms a straight diagonal line which represents the hypotenuse of Pascal's Triangle. Beginning with series $r = 2$, in the fourteenth minute column, the sum of the number in this series plus the number above it in the $r = 1$ series gives $1 + 1 = 2$, which is the

TABLE V

THEORETICAL NUMBER OF FIRST FINDINGS FOR EACH TIME-PERIOD FROM 1 TO 15 MINUTES
For Each Value of r From 1 to 14

POSITIVE MINUTE PERIODS	PERIODS OF SEARCH EXPRESSED IN MINUTES															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	WEIGHT
$r = 1$	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	3003
$r = 2$	14	27	39	50	60	69	77	84	90	95	99	102	104	105	105	429
$r = 3$	91	169	235	290	335	371	399	420	435	445	451	454	455	455	455	99
$r = 4$	364	650	870	1035	1155	1239	1295	1330	1350	1360	1364	1365	1365	1365	1365	33
$r = 5$	1001	1716	2211	2541	2751	2877	2947	2982	2997	3002	3003	3003	3003	3003	3003	15
$r = 6$	2002	3289	4081	4543	4795	4921	4977	4998	5004	5005	5005	5005	5005	5005	5005	9
$r = 7$	3003	4719	5643	6105	6315	6399	6427	6434	6435	6435	6435	6435	6435	6435	6435	7
$r = 8$	3432	5148	5940	6270	6390	6426	6434	6435	6435	6435	6435	6435	6435	6435	6435	7
$r = 9$	3003	4290	4785	4950	4995	5004	5005	5005	5005	5005	5005	5005	5005	5005	5005	9
$r = 10$	2002	2717	2937	2992	3002	3003	3003	3003	3003	3003	3003	3003	3003	3003	3003	15
$r = 11$	1001	1287	1353	1364	1365	1365	1365	1365	1365	1365	1365	1365	1365	1365	1365	33
$r = 12$	364	442	454	455	455	455	455	455	455	455	455	455	455	455	455	99
$r = 13$	91	104	105	105	105	105	105	105	105	105	105	105	105	105	105	429
$r = 14$	14	15	15	15	15	15	15	15	15	15	15	15	15	15	15	3003

next number on the left (thirteenth column) of the $r = 2$ series. The expanded number 2, plus the one above in the same column gives 3, which is the next number to the left (twelfth column) in the $r = 2$ series, etc., to the first column. Series $r = 3$ is similarly expanded from right to left, and so on until all the series are expanded to $r = 14$. These numbers from left to right represent the increase in positive findings for each additional minute of time for each series. Beginning with the column for the first minute and adding the increase for the second minute the value for the two-minute period is obtained, by adding the increase for the third minute the value for the three-minute period is obtained, and so on throughout each series. These figures are set out in Table V.

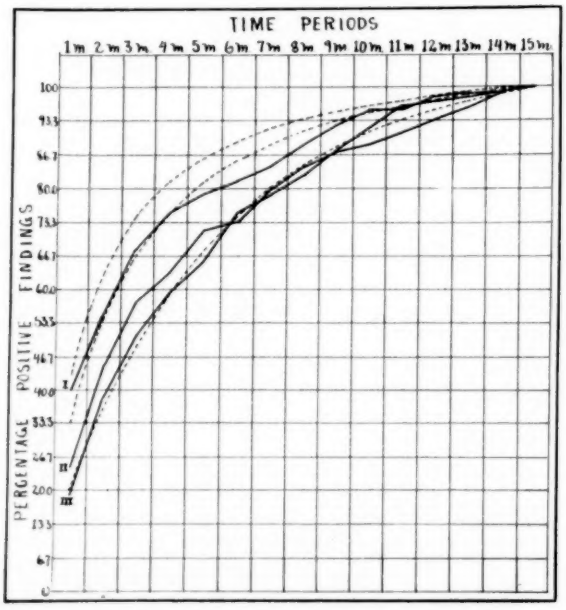

Fig. 1.—Chart showing efficiency curves of Groups I, II, and III in heavy solid lines from percentages given in Table III. The theoretical mate for each curve starts immediately above it as a light broken line.

It is fair to assume that any unknown preparation of rare bacilli is equally likely to show any number of positive minutes from one minute to fourteen minutes. Therefore, each series would have the same weight in any combination of one or more series; but the number of combinations for each series varies from 15 to 6435. It is evident then that the series with a small number of combinations would be repeated in practice many times before the series with the larger number of combinations would be exhausted. The theoretical number of such combinations for each series 1 to 14, would be represented by the least common multiple of the respective number of combinations, which is 45045. Division of this number by the number of combinations for a series gives the weight for that series. These weights are given in Table V, opposite each series.

Multiplying each number of a series (Table V) by its weight and adding these weighted values in the vertical columns gives the combined weighted values for any combination of series desired. From these figures are derived the theoretical percentage findings for each time-period (Table VI). The number of series chosen for each group was selected to conform to the averages found in experience. Referring to Table II the average number of positive minute periods for each group is 6.3, 5.0, and 3.3 respectively, which corresponds closely to the sum of weighted series of combinations from 1 to 12, 1 to 9, and 1 to 5, yielding averages of 6.5, 5.0 and 3.0. These percentage values are plotted on the chart (Fig. 1) in fine broken curves. The heavy solid curves represent the data from Table III.

TABLE VI

COMBINED THEORETICAL PERCENTAGE FINDINGS FOR EACH TIME-PERIOD UP TO 15 MINUTES

PERCENTAGE VALUES OF COMBINED WEIGHTED SERIES	MINUTE PERIODS														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 to 5	20	36.2	49.2	59.7	68.1	74.8	80.2	84.5	88.5	90.9	93.3	95.4	97.2	98.7	100
1 to 9	33.3	54	67	75.6	81.5	85.7	88.9	91.1	93.3	94.9	96.3	97.4	98.4	99.2	100
1 to 12	43.3	64	75	81.7	86.1	89.3	91.7	93.5	95	96.2	97.2	98.1	98.8	99.5	100

A study of the chart shows fair approximation of the experience curves to their respective theoretical curves. The curve of Group III almost coincides with its theoretical curve for the greater part of its course. Curves of Group I and Group II fall below their respective theoretical curves throughout, which is explained by the fact that there was a conscious selection of detailed records to be made. Occasionally a preparation showed sufficient bacilli for a safe diagnosis in the early minutes, so that a fifteen-minutes' search was not made. The inclusion of these records would raise the curves in the early minutes.

As the main purpose of this study is to ascertain the desired amount of time one should spend in searching for rare bacilli, it should be noticed that the first-minute period both in theory and experience gives very low percentage results. The theoretical and experience curves of Group III yield about 20 per cent of the possible diagnoses for the full fifteen-minute period. As the technic employed for this group was essentially the same as Kinyoun's, it is evident that he overlooked the importance of the time of search. All methods yield at least 65 per cent of possible diagnoses within a five-minute period and at least 88 per cent in a ten-minute period. It is interesting to note that the method (Group III) giving the more perfect distribution, secures less percentage result in the early minutes. In fact there are many individual specimens in the records in Group I which with thorough distribution would have shown bacilli through each minute period and would have been excluded from tabulation. The average number of bacilli, free and in groups in Group I (Table II), is 19.5, which should furnish more than one bacillus for each minute's search. There were 47 preparations of 110 in Group I showing from 15 to 114 bacilli in fifteen minutes; 57 of 200 in Group II with

15 to 155 bacilli; but only 6 of 110 in Group III with 15 to 38 bacilli. Exclusion of these records would lower the curves of Groups I and II for the early minutes. Table II shows almost complete disappearance of groups of bacilli in Group III. This is attributable, as I have shown,[5] to the resolution of groups of bacilli into their component cells, by the solvent action of the hydrocarbons upon the interbacillary wax.

Elsewhere I have shown[6] that working with sputum shaken by mechanical shaker, the chances of making a positive diagnosis are practically exhausted during the first fifteen minutes' search; 120 sputa, negative for fifteen minutes, were searched for a second fifteen-minute period, with only 3 diagnoses resulting. Each diagnosis required an expenditure of ten solid hours' work. Such a result does not justify the time consumed. The law of diminishing returns applies here as in economics.

CONCLUSIONS

1. There is a fairly definite efficiency value for each period of time in searching for rare tubercle bacilli in stained preparations.

2. This value varies within rather narrow limits, depending directly upon the degree to which uniform distribution of bacilli is attained by the various methods of preparation. In general the method securing the best distribution gives the least percentage value up to a ten-minute period of search.

3. In the direct smear preparations of rare bacilli, a search for five minutes yields about 80 per cent of positive diagnoses obtainable in a fifteen-minute search. With methods securing uniform distribution, as with the mechanical shaker with or without enrichment, a 90 per cent positive result is obtained in ten minutes. Any time period under five minutes seems inadequate for any method.

4. For careful work and especially in comparing the efficiency of one method with another a ten-minute or better a fifteen-minute period should be employed.

5. With good distribution of bacilli, a longer search than fifteen minutes is not justified by the additional results secured.

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THE STANDARDIZATION OF ACIDS AND ALKALIES*

By Reginald Stuart Hunt, Ph.D., Boston, Mass.

IN EVERY clinical and chemical laboratory there is a constant need for standard acid and alkali solutions for use in a large variety of titrimetric procedures. The usual method is to prepare a standard acid solution the strength of which is determined against weighed portions of such pure reproducible materials as sodium bicarbonate, calcium carbonate, or similar alkaline substances. The alternative is the temporary standardization of the alkali solution by weighed portions of a solid acid, such as oxalic or certain of the acid salts. Such standardization operations must be repeated at fairly frequent intervals and in both cases the acid serves as the continuous standard.

Some years ago in a paper on thermochemical procedures, Richards and Rowe[1] described an analytical method in which the alkali constituted the continuous standard solution. In their hands the method evidently gave highly satisfactory results and I adopted it a number of years ago and have since used it continuously. Feeling that it offers advantages over the conventional procedures, a brief restatement of the method is here offered particularly for the use of those laboratories where acid-alkali titrations are routine operations.

The method can be described, in brief, as the preparation of a carbonate free solution of sodium hydroxide, the equilibration of this with a hydrochloric acid solution of highest purity and the determination of the acid strength of the latter by accurate silver analyses. This permits the expression of the alkali strength in terms of silver. By preserving the integrity of the alkali solution, which is the crucial factor, a permanent and readily available standard is thereby secured. The details may be given as follows:

Preparation of Alkali.—1. Container. For the permanent storage of the alkali, a paraffined lined bottle is to be used. This is furnished with a two-hole rubber stopper, one hole of which carries a short glass tube connected with a long soda-lime column for the ingress of air, while in the other hole is placed a glass delivery tube, on the lower portion of which, inside the bottle, is blown a small glass bulb with a small orifice on the superior face. Under ordinary conditions this tube is raised so that it is out of contact with the liquid, and only comes in contact when a sample is to be withdrawn. The appended sketch (Fig. 2) and later description will make this entirely clear. The paraffined-lined bottle is prepared as follows: The container, preferably a bottle of the X X tincture type, is thoroughly cleaned—first, with bichromate solution, and then with distilled water. It is then thoroughly dried. Hard paraffin (paro-wax is very good for this purpose) is melted up on the basis of about one pound of paraffin for a four liter bottle. After complete melting, the wax is

*From the Evans Memorial, Boston, Mass.
Received for publication, December 19, 1930.

heated some twenty or thirty degrees above the melting point. A large porcelain casserole is used for this purpose. While the wax is melting, the operator, having put on a heavy pair of leather gloves, warms up the entire bottle by rapid rotation in a large naked flame to a temperature approximating the final temperature of the wax. The wax is then poured carefully into the bottle, the glass stopper inserted, the bottle inverted and slowly rotated. Do not shake at any time, as this causes bubble formation. As the bottle and wax slowly cool, a deposit of solid wax forms in successive layers around the neck and shoulders of the bottle. When the coating is of adequate thickness, the bottle is tipped more on the side and gradually the sides are encased by slow rotation with a uniform layer of paraffin. The last step is to place the bottle in an erect position and allow the base to become thoroughly coated. The stopper is then quickly withdrawn, which breaks the paraffin seal at the neck. The excess of still fluid paraffin is poured out, and the still soft paraffin at the neck of the bottle is brought up into the neck orifice with the finger. Hot paraffin is next painted on to the uncovered surfaces of the neck until level with the opening. The rough surface thus presented is smoothed by warming the nipple of the stopper, dropping it into the neck, rotating the stopper rapidly and withdrawing. The hot surface melts and homogenizes the paraffin coating. Later on, when the rubber stopper is inserted, it can be painted with paraffin externally.

Purification of Alkali.—2. Having decided on the concentration to be used, enough alcohol purified sodium hydroxide is dissolved in pure distilled water to give perhaps a 15 per cent excess of alkali. After solution and thorough homogenizing by continued shaking, samples are withdrawn for a carbonate analysis. The best practice here is to use a volumetric pipette, dissolve the sample in 100 c.c. of pure water, chill the mixture to 0 degree, and then titrate with cold hydrochloric solution of approximately equal strength,* first using phenolphthalein as an indicator, and completing the titration with methyl orange. The first titration gives roughly all of the hydroxide and half of the carbonate: the second, the residual carbonate. The difference of the two titrations multiplied by two and divided by the total amount of acid necessary for complete neutrality gives the percentage of sodium present as carbonate. Crystallized barium hydroxide, $Ba(OH)_2.8\,H_2O$, is then dissolved in pure distilled water at the boiling temperature and added; enough is used to precipitate all of the carbonate, and leave approximately a 10 per cent excess over the amount calculated for exact precipitation. The barium carbonate which forms settles out, and overnight will condense to a heavy layer at the bottom of the bottle.

To transfer the supernatant liquid the following device is used. A soda lime train (Fig. 1) is connected through a three-way glass stopcock, both with the bottle containing the solution and that empty bottle to which the carbonate-free liquid is to be transferred. Similarly the bottle containing the solution is connected with the empty bottle, and the rubber stopper in the

*Unanalyzed acid may be used, as the computation depends solely on the ratio of the two volumes used.

latter carrying these two tubes has also a third hole which permits connection with a suction device. By arranging the stopcock properly air is sucked through the soda lime, through the connecting tube or by-pass and delivered at the bottom of the empty bottle (*A*). In this way the air in the bottle is swept out by carbon dioxide-free air. When the operation is completed, the delivery tube into the empty bottle is raised so that the outlet is at the top instead of the bottom, and then the three-way stopcock is turned so that suction takes place, through the full bottle, transferring the liquid in an atmosphere free from carbon dioxide (*B*). Only as much of the liquid is carried over as can be removed without disturbing the precipitate of barium carbonate. To aid this the lower end of the outlet tube is turned upward so that the current flows from above and not from the layer of precipitate. When transference is complete, the rubber stopper is rapidly removed and replaced with the glass stopper; aliquot portions of the carbonate-free solution are then removed and the barium content determined as $BaSO_4$ by several gravimetric

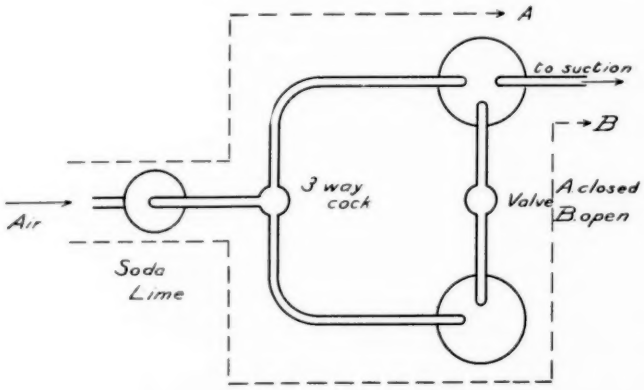

Fig. 1.

analyses. To the solution is next added the amount of a very pure sodium sulphate solution calculated to precipitate the barium. After thorough homogenizing, and allowing ample time for the barium sulphate to settle (standing overnight), the clear supernatant carbonate- and barium-free solution is then transferred to the paraffin-lined stock bottle by the method just described. The alkali solution is now complete, and will remain unchanged for years, if proper precaution be exercised during storage and the removal of samples.

Preparation of Acid.—The purest hydrochloric acid of substantially 38 per cent strength is diluted with an equal volume of pure distilled water. The acid, now approximately 20 per cent, is placed in a Harvard distilling flask with constricted neck. A transparent quartz condenser is inserted in the constriction and the mixture is distilled, exercising precautions against contamination of the distillate by dust, by the use of a hooded adapter. The middle third portion is reserved and this is redistilled, again collecting the middle third portion. This very pure acid, of approximately 20.2 per cent is then diluted with the proper amount of pure distilled water which has been freed from carbon dioxide by boiling, with subsequent cooling in a carbon dioxide-free

atmosphere, then placed in a resistance* glass bottle, and homogenized by continued shaking.

Titration of Acid Against Alkali.—When the acid is prepared, samples are at once withdrawn, placed in standard type, all glass weighing burettes and, at the same time, samples of the alkali are placed in similar weighing burettes, and the ratio of the two solutions is determined by titration, using methyl red as an indicator. All diluent water must be carbon dioxide free, and precaution exercised to prevent grave contaminations during the titration. These analyses standardize the alkali. If, for any reason, other indicators come in question, parallel groups of titrations may be carried out using them, and in all final records of the acid-alkali-ratio, the indicator used should be carefully designated. Typical analyses are presented in tabular form.

TABLE I

INDICATOR	HCl (GM.)	NaOH (GM.)		RATIO
Methyl Red	29.72	21.90		1.3571
	28.37	20.90		1.3574
	32.52	23.96		1.3572
			Average	1.3572
Alizarin	28.29	20.86		1.3562
	30.06	22.16		1.3565
	29.10	21.46		1.3560
			Average	1.3562

Analysis of Acid.—The next step is to analyze the acid and this is done by the standard silver nitrate analysis, using a modification of the Harvard atomic weight technic.[2] Briefly reviewed, this consists of the dilution of weighed portions of the acid to ample volume in large glass-stoppered Erlenmeyer flasks. A slight excess of very pure fifth normal silver nitrate solution is then added slowly from a burette with steady rotation of the flask. Nitric acid of the highest purity is next added to the mixture, the stopper inserted, and the bottle shaken thoroughly for fifteen or twenty minutes, at the end of which time the silver chloride precipitate should have formed a coagulum which settles promptly from the solution after a few minutes of standing. A necessary precaution is that all particles of silver chloride shall be washed from the walls of the container into the body of the liquid. This shaking operation is repeated at irregular intervals over a period of twenty-four hours. When ready for transference, the supernatant solution is poured onto a Gooch filter and the precipitate, after repeated washings with distilled water which are also passed through the filter, is slowly washed into the crucible. The amount of wash water used is recorded and a suitable correction supplied for the silver chloride thus dissolved. After air drying the precipitate in a desiccator, it is placed in an air-bath at 100 degrees for a period of two hours and then the temperature is raised to 150 degrees for another hour. After cooling in a desiccator and weighing, heating is repeated, and the operation continued

*The earlier work was carried out in containers of nonsol glass. It is much to be regretted that this excellent material can now be obtained only under prohibitive conditions.

until the precipitate gives a constant weight. This last analysis gives the chlorine content of the freshly distilled acid, and as the chlorine and hydrogen content are initially equivalent, the strength of the alkali can be calculated directly from the silver value of the acid. Owing to the solution of the glass, the acid will change in strength in seasoned bottles by about 0.01 to 0.02 per cent per month. The alkali is unchanged.

Removal of Standard Alkali From Container (Fig. 2).—As a precautionary measure the alkali should always be shaken before use, as surface evaporation and condensation tends to produce inhomogeneity. Another precaution is to keep the paraffin-lined bottle in a cool place, and away from direct sunlight, as this tends to loosen the lining.

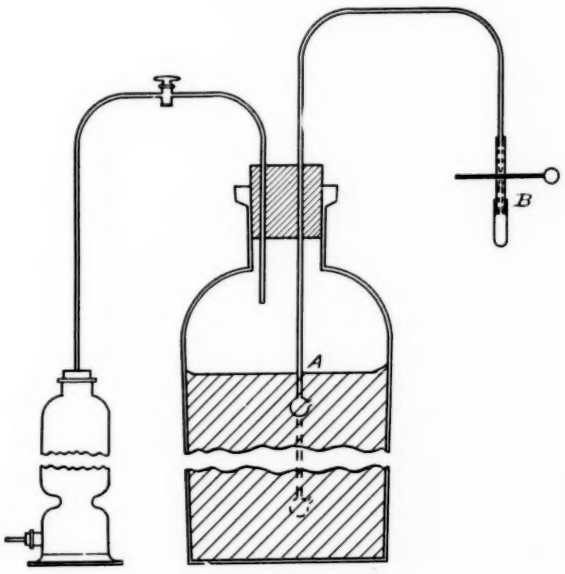

Fig. 2.

1. Force down outlet tube until tip (*A*) is below surface of liquid.

2. Open stopcock separating soda lime tower from bottle.

3. Connect small rubber bulb with bottom of soda lime train.

4. By compressing the bulb, raise the gas pressure inside the bottle.

5. Remove glass shield (*B*) from tip, open pinchcock, controlling mouth of outlet tube, and allow 20 or 30 c.c. of alkali to flow out under pressure. This rinses the tube and the solution is discarded. Next fill the weighing burette by the same method, stoppering the latter at once when full.

6. Raise delivery tube until outlet is above the surface of liquid and expel liquid trapped in tube by raising the gas pressure through the bulb and allowing it to blow off by opening the outlet.

7. Cap mouth of outlet tube.

8. Close stopcock between soda lime tower and bottle.

Titration of Other Acids.—The standardization of the alkali depends upon the equilibration of a strong base against a strong alkali, the two being of substantially equivalent strength. As the volumes used under these conditions are approximately equal, no air correction need be made for the weight of the solutions used. This is not the case, however, in subsequent analyses where the standard alkali and the acid to be determined are of divergent concentrations.

Another point to be touched on lies in the translation of the gravimetric data into volumetric relations. This is readily accomplished by continuing the use of the weighing burette with the standard solution, while the unknown which is desired for volumetric purposes, is measured from a carefully standardized burette. The weight of the standard solution gives the actual amount of acid or alkali used, and this of course, by a simple arithmetical procedure permits the calculation of the volumetric strength of the unknown.

Analyses.—A few figures will give some index both of the degree of accuracy of the method, and the permanency of the standard.

TABLE II

	HCl (GM.)	AgCl (GM.)		NaOH EQUIVALENT PER GM.
Acid I	35.1481	0.9738		0.007733
1928	35.1886	0.9742		0.007727
	36.5136	1.0095		0.007728
			Average	0.007729
Acid II	40.3182	1.0027		0.0069401
1930	40.2736	1.0019		0.0069424
			Average	0.0069413

ANALYSIS	ACID I (GM.)	NaOH		NaOH PER GM. SOLUTION
1928	29.72	21.90		0.0104888
	28.37	20.90		0.0104912
	32.52	23.96		0.0104895
			Average	0.0104895
	ACID II (GM.)			
1930	32.61	21.58		0.0104895
	28.14	18.62		0.010490
	29.14	19.28		0.010491
			Average	0.010490

So close an agreement is, of course, fortuitous. It is safe to say, however, that changes in the alkali should not exceed 0.01 per cent in a year and are usually less.

SUMMARY

A method for the accurate standardization of acid and alkali solutions is described in which the alkali is the reference standard.

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QUANTITATIVE ESTIMATION OF TOTAL PROTEIN IN CEREBROSPINAL FLUID*

A New Method With Report on 359 Cases

By James B. McNaught, M.D., San Francisco, Calif.

THE examination of cerebrospinal fluid is of great importance in the diagnosis of cases of organic disease of the nervous system. Estimation of total protein is unquestionably one of the important tests. Diagnosis is seldom based on this determination alone, but in correlation with other tests on the fluid, it may often serve to indicate which of several possible causes is the basis of the clinical manifestations.

Quantitative determination of protein in cerebrospinal fluid is justly replacing the older and less satisfactory qualitative tests of Pandy,[1] Nonne-Apelt,[2] Ross-Jones[3] and Noguchi.[4] The chief source of dissatisfaction with these qualitative tests is the difficulty in differentiating normal from abnormal quantities of precipitated protein and the still greater difficulty of recording the results in these tests for subsequent comparison in different fluids. In reducing protein determinations to numerical figures by quantitative methods normal and abnormal spinal fluids are more readily distinguished and the results of different observers may be more accurately compared.

HISTORICAL

Various methods of quantitative determinations of protein in cerebrospinal fluid have been reported. The favorite methods have been dependent upon reading the quantity of precipitated protein in a calibrated tube or comparing it with a standard of known protein concentration. Mestrezat[5] precipitated the protein with heat and trichloracetic acid and compared the unknown by diaphanometry with a series of standards of known protein content. Grey[6] similarly constructed a series of small sealed tubes containing varying amounts of albumin in 1 ml. of saline precipitated with 0.1 ml. of 25 per cent salicyl-sulphonic acid. He read the opacity produced by adding 0.1 ml. of the acid to 1 ml. of cerebrospinal fluid, against the standards. Sicard and Cantaloube[7] developed their "rachialbuminimètre" in which the protein was precipitated by heat and trichloracetic acid and the precipitate read after five hours by means of the graduations. Arnaud[8] modified this last method by precipitating with acetic acid, alcohol and carbon tetrachloride, reading after twenty minutes. Ravaut and Boyer[9] produced a turbidity by adding a sulphosalicylic acid solution to the spinal fluid and compared this with a standardized suspension of silver chloride in special tubes. Berger[10] added 1 ml. of cerebrospinal fluid to 2-3 ml. of sulphosalicylic acid solution in a test

*From the Laboratory of Clinical Pathology, Stanford University Hospital, San Francisco, California.

Received for publication, January 22, 1931.

tube, then an albumin solution of known concentration to a like amount of acid in another tube until the turbidities were the same. The quantity of albumin added to the second tube indicated the albumin content of the cerebrospinal fluid. He read the turbidities in a specially constructed colorimetric apparatus called a titrocomparator. Bloch and Pomaret[11] precipitated the protein with nitric acid and compared transparencies with standard tubes of various opacities prepared from a mixture of tincture of benzoin, gelatin, glycerin, gentian violet and formalin. These tubes were prepared so that their opacities were identical with known quantities of protein in human serum treated with nitric acid.

Denis and Ayer[12] produced a turbidity with sulphosalicylic acid and compared this in a colorimeter with a standard solution prepared at the same time from blood serum of known protein content. This method has proved very satisfactory. It necessitates only a short time for completion of the test and requires but a small amount of cerebrospinal fluid (from 0.6 to 1 ml.) providing one has available a colorimeter with small cups holding only 2 ml. Fremont-Smith and Ayer[13] described certain modifications of the Denis-Ayer technic and reported determinations on 622 fluids. Young and Bennett[14] described a method by which they precipitated the protein from 2 ml. of cerebrospinal fluid with alcohol, heat and acetic acid and read the volume of precipitate in a Hopkins vaccine tube after centrifuging for three minutes at 3000 revolutions per minute. Their results seemed to be higher than those of other workers. Recently these authors[15] corrected their former calculations and described a method similar to that reported by Shevky and Stafford.[16] This new method consisted of precipitating the fluid with Tsuchiya's reagent[17] and centrifuging for five minutes at 2000 revolutions per minute in a graduated tube in which each 0.01 ml. volume is equivalent to 10.3 mg. of protein per 100 ml. of cerebrospinal fluid.

Exton[18] announced a new optical instrument, the junior scopometer, which utilizes the extinction or vanishing point criterion to measure both color and turbidity. He reported very satisfactory correspondence in quantitative determinations of albuminous urines with this instrument and protein estimations made by the Kjeldahl method. He developed turbidity by mixing equal volumes of urine and sulphosalicylic-acid-sodium-sulphate reagent. Exton stated* that this technic for determination of protein in urine is equally adaptable to cerebrospinal fluid.

Wu[19, 20] made use of the color reaction of blood protein with phenol reagent and developed a colorimetric method of protein determination using a tyrosine solution as a standard. Ling[21] adapted this method to the determination of protein in cerebrospinal fluid. Hewitt[22] likewise applied the Wu method to the determination of total protein and various protein fractions of cerebrospinal fluid using 2 ml. for the total protein determination. Matz and Novick[23] presented methods for the determination of albumin, globulin, and total protein in cerebrospinal fluid based upon the work of Hewitt and Wu. They made quantitative estimations of globulin and total protein and obtained the albumin content by deducting the former from the latter.

*Personal communication.

NEW METHOD

Several years ago Dr. H. A. Wyckoff and Mr. G. Arguedas, of the Clinical Laboratory of Stanford University Hospital, developed a method for the quantitative determination of protein in urine which has proved more satisfactory in our hands than any other. These workers, dissatisfied with the variable results of the Purdy,[24] Shevky-Stafford[16] and other quantitative albumin tests depending upon reading the volume of precipitate after centrifugalization, found it advisable to modify the method with a view to eliminating the variable element, namely, the speed of the centrifuge. The impracticability of controlling the speed of different centrifuges has been experienced by all. In order to eliminate this it was only necessary to prepare a standard solution of protein. The essentials of their method are as follows: Read the volume of precipitate in each of two 15 ml. graduated centrifuge tubes, one containing a standard of known protein content and the other a urine of unknown protein content after both have been simultaneously precipitated and centrifuged. By means of a simple formula the quantity of protein in the unknown is expressed in grams per cent. Their standard is a 0.1 per cent casein solution. Tsuchiya's reagent[17] is used as the precipitant.

At the suggestion of Dr. Wyckoff, the writer adapted the above principle and reagents to the determination of protein in cerebrospinal fluid.

The reagents are as follows:

1. Tsuchiya's reagent:

Phosphotungstic acid	1.5 gm.
Concentrated hydrochloric acid	5.0 ml.
Ethyl alcohol, sufficient to make	100.0 ml.

2. Casein Protein Standard:

Casein, C.P.	1.0 gm.
Sodium bicarbonate	1.0 gm.
Sodium chloride	25.0 gm.
Formalin	20.0 ml.
Distilled water, sufficient to make	1000.0 ml.

Place the casein and sodium bicarbonate in a beaker with about 100 ml. of distilled water. Boil for one minute, stirring constantly, cool, and transfer to a 1000 ml. volumetric flask, wash the beaker with several quantities of water adding the washings to the flask. Add the sodium chloride and formalin. Bring the volume up to the mark with water. Mix and keep in a well-stoppered bottle at room temperature. This solution keeps well and is equivalent to a 0.1 per cent albumin solution (within 0.1 per cent) as checked by Kjeldahl and nonprotein-nitrogen determinations.

The necessary equipment:

1. Centrifuge tubes accurately graduated to 0.01 ml. in the cylindrical tip so that readings may be made in the third decimal place. (Fig. 1.)*

*These tubes were prepared by Arthur H. Thomas Co., Philadelphia, Pa.

2. Glass stirring rods prepared by sealing over the small ends of capillary pipettes.

3. Capillary pipettes.

4. Test tube rack.

5. Centrifuge.

The method:

1. Place the protein standard into the centrifuge tube marked S (standard) accurately to the 1 ml. mark by means of a capillary pipette.

2. Place the cerebrospinal fluid similarly into the tube marked U (unknown) to the 1 ml. mark.

3. Add Tsuchiya's reagent to the 2 ml. mark in each tube.

Fig. 1.—Accurately graduated centrifuge tube with cylindrical tip.

4. Mix thoroughly with a small glass stirring rod in each tube.

5. Balance the tubes against each other.

6. Centrifuge for three to five minutes at a speed sufficiently rapid to obtain a firm packing of the precipitate.

7. Read the volume of the precipitate in each tube against a dark background.

8. If the volume of the unknown precipitate is markedly greater than that of the standard the test should be repeated from the beginning after diluting the cerebrospinal fluid 2, 3, or 4 times with 2.5 per cent sodium chloride solution in order to have the readings of the two volumes within reasonable range of each other. This dilution is taken into consideration in the calculations.

9. Calculations. Formula: $U\% = \frac{RU}{RS} \times S\% \times D.$

U% = per cent of protein in unknown.
RU = volume of the precipitate of the unknown fluid.
RS = volume of the precipitate of the protein standard.
S% = per cent of protein in the standard.
D = dilution.

Example:

RU = 0.048 ml.
RS = 0.060 ml.
S% = 0.1.
D = 3.

$$U\% = \frac{0.048}{0.060} \times 0.1 \times 3 = 0.24 \text{ gm. \% or } 240 \text{ mg. per } 100 \text{ ml.}$$

If the protein content of the cerebrospinal fluid is nearly normal no dilution is necessary.

Any number of unknown fluids may be examined at the same time depending upon the capacity of the centrifuge carrier but there must always be a standard tube in each set. Most centrifuge carriers accommodate 4 or 8 tubes which means that 3 or 7 unknowns may be examined at one time. The readings of each set of tubes of 4 or 8 must be calculated according to the reading of the standard in its own set. By this method it is easy to make 7 unknown determinations in fifteen minutes.

The tubes are easily cleansed by means of a capillary pipette fastened to a water faucet suction pump, rinsed several times with water, then alcohol and ether.

EXPERIMENTAL OBSERVATIONS

In order to ascertain the variation in readings dependent upon the length of time elapsing between precipitation and centrifugalization several series of tubes were set up half containing protein standard and half containing cerebrospinal fluid. All were precipitated at the same time and pairs of tubes (standard and unknown) were centrifuged at intervals varying from three to thirty minutes from the precipitation time as accurately as possible at the same speed and for the same length of time. The results showed that the length of time allowed for complete precipitation is of no practical importance inasmuch as both the standard and the unknown fluid apparently precipitate in the same ratio. It did show that the precipitating reagent should be added to both the standard and the unknown as nearly simultaneously as possible.

It is realized that an inherent error exists in any method which depends upon the precipitation of colloids, the physical state of which will obviously vary in different experiments. Actual multiple precipitations of various specimens from the same standard yielded almost identical amounts of centrifuged sediment, so that the error is a small one which does not seem important in relation to the actual variation of protein in spinal fluids under various conditions.

Results obtained by this new method were compared with those of two other methods:

1. The difference between the nonprotein nitrogen readings and the Kjeldahl total nitrogen readings gives a value considered to represent the protein nitrogen. This value multiplied by 6.25 gives the protein content. Table I presents the results obtained on three cerebrospinal fluids examined by this method and by the author's method. They closely approximate each other, the percentage error being less than 5 per cent.

TABLE I

TOTAL PROTEIN DETERMINATIONS BY KJELDAHL AND AUTHOR'S METHODS

SPINAL FLUID NUMBER	TOTAL PROTEIN IN MG. PER 100 ML.		
	KJELDAHL NONPROTEIN NITROGEN METHOD	AUTHOR'S METHOD	PER CENT ERROR
1	46.2	44.6	3.1
2	48.1	46.0	4.4
3	1130.2	1148.0	1.5

2. The colorimetric method of Denis and Ayer and the author's method were used on 10 spinal fluids with the results shown in Table II. Protein determinations by the author's method were higher in some cases and lower in others. None varied to any clinically significant degree, the average percentage error being 3.7 per cent. The Denis-Ayer protein standard was prepared according to the technic of Fremont-Smith and Ayer[13] so that it contained 30 mg. of protein per 100 ml. Using this standard as an unknown, several protein determinations were made upon it at different times by the new method. The average percentage error was 1.3 per cent.

TABLE II

TOTAL PROTEIN DETERMINATIONS BY DENIS-AYER AND AUTHOR'S METHODS

SPINAL FLUID NUMBER	TOTAL PROTEIN IN MG. PER 100 ML.		
	DENIS-AYER METHOD	AUTHOR'S METHOD	PER CENT ERROR
1	27.7	28.5	2.8
2	62.0	59.0	4.9
3	36.5	35.4	3.1
4	45.4	44.6	1.8
5	39.0	41.0	5.1
6	28.9	30.0	3.8
7	48.0	45.9	4.4
8	66.0	62.8	4.9
9	78.0	75.0	3.9
10	943.0	967.0	2.5

CLINICAL OBSERVATIONS

The above-described method for determining the total quantity of protein has been used upon available cerebrospinal fluids for a year and a half. Altogether more than 1,000 determinations have been made upon 590 patients, 359 of these have been selected for use in Table III. Only those cases were chosen whose history, symptoms, physical signs, laboratory, operation or autopsy findings permitted a fairly accurate diagnosis. One reading is tabulated for each patient although many of them had several readings. The skeletal outline used in Table III follows the general scheme of Ayer and Foster[25] in reporting 429 cases. All readings are on lumbar fluids unless otherwise indicated.

TABLE III

TOTAL PROTEIN LEVELS IN NORMAL AND PATHOLOGIC CEREBROSPINAL FLUIDS BY THE AUTHOR'S METHOD

	NUMBER OF CASES	MG. PROTEIN PER 100 ML.		
		HIGH	LOW	AVER.
I. *Normal*	23	40	17	29
II. *Syphilis of Central Nervous System:*				
1. Tabes:				
A. Active:				
a. Untreated	5	79	48	62
b. Treated, tests still positive	36	101	30	51
B. Active with general paresis gold solution curve:				
a. Untreated	5	155	57	92
b. Treated, tests still positive	11	84	38	53
C. Inactive, symptoms stationary, tests essentially negative	8	45	24	32
2. Paresis:				
A. Untreated	4	116	78	90
B. Treated, tests still positive	36	110[a]	29	60
3. Taboparesis, treated	12	60[b]	23	45
4. Optic atrophy:				
A. Untreated	2	70	46	58
B. Treated, tests essentially negative	3	44	31	37
5. Cerebrospinal Syphilis:				
A. Meningitic type, average 216 cells	4	161	85	122
B. Active:				
a. Untreated	2	90	33	61
b. Treated, tests still positive	33	134	30	52
C. Inactive	9	52	30	43
III. *Nonneurologic Syphilis:*				
1. Tertiary, active, blood positive:				
A. Untreated	6	45	26	35
B. Treated	39	62	13	40
2. Inactive, blood negative	15	59	23	39
3. Congenital, under fourteen years of age:				
A. Untreated	4	45	19	28
B. Treated	4	36	20	31
IV. *Vascular Hypertension:*				
1. Symptoms of old cerebral lesions	10	96	27	55
2. Symptoms of recent cerebral lesion, four days	1			101
V. *Encephalitis, inactive clinically*	2	45	41	43
VI. *Poliomyelitis, acute*	5	107	65	89
VII. *Meningitis:*				
1. Epidemic, meningococcus	7	967	111	581
2. Streptococcus	1			174
3. Tuberculous	4	615	60	254
VIII. *Degenerations of Central Nervous System:*				
1. Paralysis agitans	6	58	40	48
2. Multiple sclerosis	7	75	28	53
Cisternal fluid	1			25[c]
3. Combined sclerosis	1			35
4. Combined sclerosis with pernicious anemia:				
A. Active	2	46	46	46
B. Remission	2	34	26	30
5. Amyotrophic lateral sclerosis	2	32	26	29
6. Infantile spastic hemiplegia	1			21
7. Microcephalic idiot	1			34
8. Mongolian idiot	1			25
9. Mental deficiency	2	28	21	25

TABLE III—CONT'D

	NUMBER OF CASES	MG. PROTEIN PER 100 ML.		
		HIGH	LOW	AVER.
IX. *Brain and Cord Tumors and Compressions:*				
1. Neuroma, left acoustic	1			33
2. Fibrosarcoma, left sphenoid	1			500
3. Adenoma, pituitary	1			50
4. Glioma, base of brain	1			50
5. Gliosarcoma, right frontal lobe	1			140
6. Myeloma, base of skull	1			72
Ventricular fluid	1			4
7. Spongioblastoma, right frontal lobe				
Ventricular fluid	1			21
8. Arteriovenous fistula, right parietal	1			28
9. Carcinoma, extradural compressing cord at thoracic 11-12	1			746
10. Tuberculoma, extradural compressing cord at thoracic 3-4	1			1000
X. *Mental Disorders:*				
1. Dementia precox	1			37
2. Anxiety neuroses	2	35	25	30
3. Posttraumatic neuroses	1			38
4. Manic depressive psychoses	1			27
5. Acute mania	1			24
6. Alcoholic psychoses (paranoid)	1			28
7. Paranoia	2	34	30	32
8. Psychoneuroses	2	33	32	33
XI. *Miscellaneous:*				
1. Epilepsy:				
A. Unknown origin	13	62	19	41
B. Traumatic (old bullet)	1			57
2. Toxic amblyopia, wood alcohol	1			52
3. Old pachymeningitis with adhesions	2	31	28	29
4. Multiple neuritis	3	83	32	57
5. Gonococcal endocarditis	1			32
6. Sydenham's chorea	1			31

[a]One case apparently belonging to this group but undoubtedly complicated gave 510 mg. per cent.

[b]One case given 3 intravenous salvarsan injections fifteen years ago and no treatment since gave 204 mg. per cent.

[c]This cisternal fluid was from the case that gave 28 mg. per cent in the lumbar fluid.

COMMENTS ON TABLE III

1. *Normal.*—The total protein in the 23 normal lumbar fluids ranges from 17 to 40, averaging 29 mg. per 100 ml. Some of these fluids were taken from patients about to undergo operation for hernia under spinal anesthesia, the others were from patients who presented no evidence of organic or central nervous system disorders and whose lumbar fluids were normal in other respects. Table IV gives the normal total protein values for cerebrospinal fluid as reported by various observers. It is difficult to draw a sharp line between normal and abnormal quantities of protein in lumbar fluid but it appears that total protein values above 40 mg. per 100 ml. should be considered abnormal.

An insufficient number of cisternal and ventricular fluid examinations have been made by the method reported in this paper to determine their normal value. Fremont-Smith and Ayer[32] report total protein in normal cisternal

TABLE IV

NORMAL RANGE OF TOTAL PROTEIN BY VARIOUS OBSERVERS

	NUMBER OF FLUIDS EXAMINED	TOTAL PROTEIN IN MG. PER CENT	
		NORMAL RANGE	AVERAGE
Ayer and Foster[25]	48	16.0-38.0	25.0
Eskuchen[26]		20.0-30.0	
Fremont-Smith and Ayer[13]	13	20.0-40.0	28.0
Greenfield[27]		20.0-35.0	
Grey[6]	65	20.0-40.0	28.0
Hewitt[22]			30.0
Ling[21]	6	18.8-37.9	25.5
Mestrezat[28]		15.0-30.0	
McNaught	23	17.0-40.0	29.0
Penfold and Irving[29]	9	10.0-30.0	24.0
Riser[30]	50	15.0-25.0	20.0
Wilcox, Lyttle and Hearn[31]	23	30.0-80.0	52.0
Young, Bennett, Christlieb, Myers[15]		5.0-20.0	

fluids as 10-25 mg. per cent and ventricular fluid as 5-15 mg. per cent. Riser gives the normal total protein values as 15-25 mg. per cent in cisternal fluids and 5-10 mg. per cent in ventricular fluids. Various observers agree that the total protein of cisternal fluid is a few mg. less per hundred ml. than the lumbar fluid and that the quantity in ventricular fluid is still lower. The lowest total protein value obtained by the author's method was 4 mg. per cent in a ventricular fluid.

2. *Syphilis of the Central Nervous System.*—This constitutes the largest group of cases reported in this paper, numbering 170. The meningitic type, with an average cell count of 216 and giving a paretic Lange colloidal gold curve, has the highest average total protein, that of 122 mg. per cent. This value is slightly higher than that reported by others as shown in Table V. Active untreated cases of tabes with a paretic colloidal gold curve is second with an average of 92 mg. per cent. Untreated paresis is a close third with 90 mg. per cent. Matz and Novick report an average of 110 mg. per cent for their group of untreated pareties. It is noteworthy that all the highest protein values are in fluids giving a paretic type of gold curve.

TABLE V

TOTAL PROTEIN IN TREATED AND UNTREATED CASES OF SYPHILIS

	TOTAL PROTEIN IN MG. PER 100 ML.								
	TABES			PARESIS			MENINGITIS		
	RANGE	AVERAGE	NO. OF CASES	RANGE	AVERAGE	NO. OF CASES	RANGE	AVERAGE	NO. OF CASES
Ayer and Foster	22-150	78	31[a]	50-153	89	7	89-118	99	4
	29-174	54	24[b]						
Greenfield	30- 60			40-100			50-200		
Hewitt	48-139	85	6	60-226	108	17			
Matz and Novick	39- 87	62	20	63-204	110	16[a]			
McNaught	48-155	77	10[a]	78-116	90	4[a]	85-161	122	4
	30-101	52	47[b]	29-110	60	36[b]			
Ravaut and Laignel-Lavastine[33]				70- 90	80	1			
Wilcox, Lyttle and Hearn							93-113	103	2

[a] = Untreated cases only.
[b] = Treated cases only.

The treated cases all present a lower average than the untreated. Inactive cases whose symptoms are stationary and the laboratory tests on blood and lumbar fluid essentially negative have an average total protein practically within normal limits. Ayer and Foster have reported similar findings. Table V presents the tabulated protein findings of several observers on syphilitic fluids. All show higher proteins in fluids from cases of paresis than of tabes. None of the author's series show normal total proteins in untreated cases of paresis or tabes. Twenty-seven per cent of the fluids from active treated cases of cerebrospinal syphilis, 12 per cent of the fluids from active treated tabes and 8 per cent from active treated paresis give total protein values below 40 mg. per cent. These lower figures are invariably associated with cases that have received extensive treatment.

3. *Nonneurologic Syphilis.*—All of these cases had positive blood Wassermann reactions except the inactive group in which sufficient treatment had been received to render the Wassermann reaction negative. There were no signs nor symptoms of neurosyphilis. The lumbar fluids gave normal cell counts, colloidal gold curves and Wassermann reactions. The average total protein is higher than the normal average but is within normal range although individual figures are abnormally high in some cases. The inactive group does not differ materially from the active. The average of the author's 15 inactive cases is 39 mg. per cent. Ayer and Foster likewise report a high average of 34 mg. per cent for their 13 inactive cases. It is interesting that both the groups of active treated cases should average somewhat higher than the same groups untreated.

4. *Vascular Hypertension.*—This group with symptoms of old vascular accidents, cerebral lesions, hemiplegia, etc., gives an average almost twice the normal. Ayer and Foster found this group to give protein values two and a half times the normal average. The one case showing cerebral hemorrhage within four days with a total protein of 101 mg. per cent is not as high as the lowest reported by Fremont-Smith and Ayer in a series of 8 cases. Their average was 222 mg. per cent.

5. *Encephalitis.*—Both of these cases were inactive clinically and gave protein values only slightly above normal, averaging 43 mg. per cent. Fremont-Smith and Ayer reported an average of 42 mg. per cent in 3 cases. Regan[7] reported an average of 82 mg. per cent in 16 cases of active epidemic encephalitis, while Wilcox, Lyttle and Hearn reported a range of 42-248 mg. per cent in 15 active cases.

6. *Poliomyelitis.*—These 5 acute cases uniformly gave high total protein values with an average three times normal. This is in accordance with the findings of most observers except Regan who reports an average of only 30 mg. per cent in sixty acute cases as shown in Table VI.

7. *Meningitis.*—Lumbar fluids from 7 definitely proved cases of meningococcic meningitis prior to serum therapy show a total protein average of 581 mg. per cent. The one case of streptococcic type gave 174 mg. per cent.

TABLE VI

TOTAL PROTEIN IN ACUTE ANTERIOR POLIOMYELITIS

	TOTAL PROTEIN IN MG. PER 100 ML.			
	LOW	HIGH	AVERAGE	NO. OF CASES
Ayer and Foster	67	81	75	3
Fremont-Smith and Ayer	30	250	118	13
McNaught	65	107	89	5
Regan	22	85	30	60
Wilcox, Lyttle and Hearn	42	250	86	6

TABLE VII

TOTAL PROTEIN IN MENINGITIS AS REPORTED BY VARIOUS OBSERVERS

	TOTAL PROTEIN IN MG PER 100 ML.								
	MENINGOCOCCIC			TUBERCULOUS			STREPTOCOCCIC		
	RANGE	AVERAGE	NO. OF CASES	RANGE	AVERAGE	NO. OF CASES	RANGE	AVERAGE	NO. OF CASES
Ayer and Foster	69- 923	364	5[a]	68- 252	150	10			
Fremont-Smith and Ayer[11]	138- 380	220	11[a]	63-1142	244	68			
Greenfield and Carmichael[35]	100- 400			30- 400			100-200		
Grey	350- 630	485	4	40- 150	84	27			
Hewitt				93- 226	143	5			
Ling		278	1	93- 260	148	6			
McNaught	111- 967	581	7	60- 615	254	4		174	1
Penfold and Irving				40- 300	174	11			
Regan				10- 880	264	34			
Wilcox, Lyttle and Hearn	159-2600			80-1000		24	192-318	270	2

[a] = Meningococcic, streptococcic and pneumococcic meningitis grouped together.

Four cases of tuberculous meningitis average 254 mg. per cent. Table VII contains the total protein values in meningitis as reported by various observers.

8. *Degenerations of the Central Nervous System.*—Paralysis Agitans: These six cases show an average above normal which is in accordance with the reports of other investigators.

Multiple Sclerosis: The protein content is normal in only one of the seven cases. The range is not as marked as in the cases reported by some others in Table VIII.

TABLE VIII

TOTAL PROTEIN IN MULTIPLE SCLEROSIS

	TOTAL PROTEIN IN MG. PER 100 ML.			
	LOW	HIGH	AVERAGE	NO. OF CASES
Ayer and Foster	15	108	48	19
Fremon-Smith and Ayer	20	80	43	16
Matz and Novick	66	140	104	4
McNaught	28	75	53	7
Penfold and Irving	10	20	15	2

Combined Sclerosis: The single case shows a total protein within normal limits. In the four cases with pernicious anemia, it is interesting to note that the fluids taken from the patients in the active stage of the anemia are higher than those taken during a remission. Fremont-Smith and Ayer reported an average of 58 mg. per cent in 8 cases of pernicious anemia with ataxic paraplegia.

The remaining cases of this group show normal protein readings in the lumbar fluids which were also normal in all other respects.

9. *Brain and Cord Tumors and Compressions.*—The few cases in this group do not permit comparison. Only two cases show normal protein values. The high protein in the two cases of cord compression is as expected.

10. *Mental Disorders*—The eleven cases in this group show normal cell counts, colloidal gold curves, Wassermann tests and the total protein is within normal limits. Ravaut and Laignel-Lavastine reported eight cases of dementia precox as averaging 21 mg. per cent of total protein using the Ravaut-Boyer method. Bowman[36] reported the total protein for 20 cases of dementia precox by the Denis-Ayer method as varying from 18-53 mg. per cent with an average of 27.

11. *Miscellaneous.*—Epilepsy: In 13 of these cases there was no known pathologic lesion. Fifty-three per cent give total protein findings below 38 mg. per cent. Table IX shows the findings of other observers in idiopathic epilepsy.

The remaining cases of this group are too few for comment.

TABLE IX

TOTAL PROTEIN IN IDIOPATHIC EPILEPSY BY VARIOUS OBSERVERS

	TOTAL PROTEIN IN MG. PER 100 ML.			
	LOW	HIGH	AVERAGE	NO. OF CASES
Ayer and Foster	17	50	29	11
Fremont-Smith and Ayer	17	200	43	27
McNaught	19	62	41	13
Osnato, Killian, Garcia, Mattice[37]	32.9	99.6	55.6	49
Penfold and Irving	20	50	38	5

SUMMARY

1. It is felt that quantitative protein determinations on cerebrospinal fluids should be carried out routinely as an aid to diagnosis.

2. A rapid, simple, volumetric method for the quantitative determination of the total protein content of cerebrospinal fluid is described.

3. The principle is the precipitation of protein by Tsuchiya's reagent, centrifugalization in a finely calibrated centrifuge tube and comparison with a similarly treated standard protein solution.

4. The total protein in 23 normal lumbar fluids varied from 17 to 40 mg. per cent with an average of 29.

5. A table containing the total protein determinations of 359 selected cases is presented.

6. Total protein values of different observers in various pathologic conditions are tabulated.

I am greatly indebted to K. T. Liu, of the Biochemical Laboratory, for technical advice and to Miss R. M. Davis, of the Serological Laboratory, for placing so many cerebrospinal fluids at my disposal.

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Since this paper was written W. G. Exton and A. R. Rose published an article, "Clinical Determination of the Albumin Globulin Ratio in Spinal Fluid," in the Journal of the American Medical Association **96**: 36, 1931. They make a total protein determination and a globulin determination on the spinal fluid by means of a scopometer and obtain the albumin reading by subtracting the two.

CLINICAL HEMOGLOBINOMETRY

By H. E. Harvey, D.D.S., Phar.D., F.A.S.A.,* V. H. Traxler, D.D.S., B.S., M.A., and R. L. Wright, Naval Hospital, Norfolk, Va.

IN THE course of a clinical study[1] in which accurate estimation of the hemoglobin-content of the blood was essential, discrepant results obtained in using different methods led us to undertake the injury of which this paper is a report.

While there probably is little doubt among authorities that the Van Slyke and Wong chemical methods are accurate and reliable in representing the hemoglobin-content of the blood, these methods are not applicable in clinical practice because of the special apparatus and trained workers required, and the time necessary for their performance. Consequently, our inquiry was confined to an examination of the several inexpensive and relatively simple forms of apparatus in general use, the instruments actually studied being the Talqvist, the Sahli (old and new), and the Newcomer.

There is a copious literature concerning the various methods of hemoglobin determinations.

Todd's text[2] states that "There are many methods, but none is entirely satisfactory. * * * records of hemoglobin estimations are generally untrustworthy," and "A reading of 100 upon the Sahli instrument should, according to the makers, correspond to 17.3 gm. of hemoglobin in 100 c.c. of blood. * * * The normal amount of hemoglobin was formerly given as about 14 gm. per 100 c.c. of blood, but recent work has shown this to be too low for adults. The general average for both sexes during adult life appears to be about 16 gm."

Referring to the Sahli hemometer, Haden[3] says: "The standard tube as supplied with the instrument is seldom accurate." Haden,[4] as the result of rather extensive experimental work, concludes that 15.6 gm. per 100 c.c.

*All U. S. Navy.

Received for publication, February 12, 1931.

should be considered as 100 per cent for the normal adult standard. He used the Van Slyke method in checking the erythrocyte count of 52 normal persons and believes the amount of hemoglobin per cell varies little,[5] the difference being due to variation in the number of red cells. The final average reached was 15.6 gm. per 5,000,000 red cells.

Haden[5] states "There is really only one available method by which accurate quantitative determinations can be made. This is the oxygen capacity method of Haldane which may be most conveniently employed in the blood gas apparatus of Van Slyke. * * * Haldane and Smith (11)[6] working with only twelve normal individuals, found the average oxygen capacity to be 18.5 c.c. per 100 c.c. blood. This corresponds to 13.8 gm. of hemoglobin per 100 c.c. since 1 gm. of hemoglobin combines with 1.34 c.c. of oxygen. Neither Haldane and Smith nor Williamson determined the red cell count of the individuals on whom the hemoglobin determinations were done."

Stitt[7] gives: "Sahli . . . 17.2 gm. per 100 c.c." and states: "Newcomer has modified the Sahli acid hematin method by substituting for the standard solution a colored glass disc which may be used with any colorimeter made on the Duboscq pattern. Employing the Van Slyke oxygen capacity method. Myers recalibrated one of these discs and found it accurate to 1 per cent. * * * In the Newcomer haemoglobinometer the normal 100 per cent is equivalent to 16.92 gm. of haemoglobin per hundred cubic centimeters found by Williamson[8] to be normal for adult men."

Lindsay, Rice, and Sellinger[9] made the following determinations:

Sahli-Leitz	100%	= 15 gm. per 100 c.c.
Dare	109%	= 15 gm. per 100 c.c.
Newcomer	88%	= 15 gm. per 100 c.c.

and stated that a hemoglobin content of 15.6 gm. per 100 c.c. is generally accepted as normal. The Newcomer method with standardized color plate was recommended as the most accurate for clinical work. The Sahli and Dare methods were found useless without standardization.

The result of a perusal of the literature upon the subject of hemoglobin estimations leaves the reader in some doubt concerning the reliability of methods which can be employed in practice. Inasmuch as the clinical methods for determining the amount of hemoglobin are based on a known chemical reaction (. . . on a colorimetric standard. . ???), and differ from one another essentially only in respect to the amounts of hemoglobin in grams adopted by each as "100 per cent," it should be a comparatively simple matter to ensure reliable findings by using two or more methods, once the correspondences of the several instruments were established.

In order, then, to learn the corresponding points on the different scales, comparative readings were made, using a new standardized Newcomer, considered by most authors the most reliable instrument for clinical work, a new Sahli, an old Sahli, and a Talqvist.

It was soon apparent, however, that the three methods could not be correlated when using their several standard values as set forth in textbooks and by the manufacturers. The Sahli seemed to agree fairly well with the red

cell counts, so that, if credence is to be placed in the statements of Haden[10] and others as to the relationship which they found to exist in normal individuals between the number of red cells and the hemoglobin content, the Newcomer and the Talqvist (which were in disagreement) were both in error.

After eight months of experimental work, during which 478 comparative hemoglobin estimations were made, it became evident that it was useless further to attempt to correlate the findings of the Newcomer, Sahli, and Talqvist methods. The main reliance, of course, was placed on the Newcomer and Sahli* readings. Both being chemical in nature, it was not seen how a discrepancy could exist between them unless one or both of the appliances were at fault.

The Newcomer was therefore returned to the manufacturer for inspection and the following report was received: "* * * who gave this his personal inspection reports that he found nothing wrong with it except that the zero setting on the drum was off approximately 3 mm. on the circumference so as to cause a minus error of approximately 7 per cent on a normal blood solution." An adjustment was made and tests were conducted by the manufacturer before the instrument was returned to us.

When the adjusted instrument was used, it was found that the readings obtained were far different. However, individuals with red cell counts approximating 5,000,000 failed to give hemoglobin readings at the 100 mark on the Newcomer, set as normal (after Williamson[8]) to represent 16.92 gm. per 100 c.c. As a matter of fact, the average reading on the Newcomer scale for 5,000,000 red cells was found to be about 81.5. With blood of the same individuals, taken at the same time, the Sahli readings were 100. Manifestly an error was present if the Sahli standard were 17.2 gm. as set forth in textbooks, because a content of 17 gm. plus should give a Sahli reading of 122.9 if the Newcomer were correct. The following estimations on the same specimens of blood were made in 1926-27 and illustrate the discrepancy of readings at that time:

"A" Sahli scale readings.

"B" Newcomer scale readings.

"C" Transposition of the Newcomer readings into Sahli equivalents (possible after correction of instruments and formulation of correlation table).

"A"	"B"		"C"
Sahli	Newcomer		(Sahli equivalent of Newcomer readings)
95	99.0	=	122
92	86.5	=	106
92	91.5	=	112
90	91.5	=	112
85	90.5	=	111
84	87.5	=	107
80	96.0	=	117
80	78.0	=	95.5
78	86.5	=	106
67	65.0	=	81

*Experimental work demonstrates that if the carbon dioxide in the breath is allowed to enter the mixture of hydrochloric acid and blood a lower Sahli reading is obtained. A decrease as much as 10 per cent has been observed under such conditions. This change takes place rapidly and may occur upon exposure to the CO_2 for one or two seconds.

The hemoglobin-estimations below were made in 1927-28 after correction of the instruments, with the use of the correlation table for equivalent values.

"A"	"B"		"C"
Sa	Newcomer		(Sahli equivalent of Newcomer readings)
108	88	=	108
106	86	=	106
105	84	=	103
104	86	=	106
104	86	=	106
100	82	=	101
97	78	=	96
94	77	=	95
72	58	=	73
45	39	=	46

In order to ascertain whether the standard of the Sahli hemometer had been changed, as appeared very probable, we addressed an inquiry (1927) to the American representative of the manufacturer, informing him that our findings indicated a standard of approximately 13.8 gm. of hemoglobin per 100 c.c. to be the true Sahli standard. The dealer was unable to furnish the information and forwarded the inquiry to Germany. In due time word was received that 14.0 gm.* had been made standard for the nonfade glass standards. We also were informed that in the future all Sahli instruments would be standardized by having the amount in grams etched on the tubes at the corresponding percentages. This has been done. It is a matter of choice whether hemoglobin-content is estimated in grams per 100 c.c. of blood or in percentage of normal amount.

As soon as the errors in each instrument had been ascertained and calibrated, it was a simple matter to work out a table of correspondences. Thereafter, equivalents being known, the findings obtained by the Sahli and the Newcomer were practically identical. Some red cell counts and hemoglobin-values after correction of instruments:

Date	Red Cells	Hemoglobin	
10/26/27	2,880,000	54	
10/31/27	5,570,000	112	
11/10/27	3,710,000	72	
11/23/27	2,220,000	44	(8:30 A.M., patient next below)
11/23/27	2,280,000	46	(1:30 P.M., patient next above)
11/22/27	4,300,000	72	(Oligochromemia)
1/16/28	5,030,000	101	
3/19/28	4,340,000	86	
3/21/28	4,080,000	80	
4/20/28	4,700,000	94	
5/11/28	4,330,000	86	

*Excerpt from the reply of the manufacturer of the Sahli hemometer, "A specimen of blood which according to the Sahli-Leitz Haemometer 'Non-fade' shows a hemoglobin contents of 100 per cent yields the result that within 100 c.c. blood fourteen grams of pure haemoglobin is contained." Thus, the Sahli instrument may have been calibrated by the manufacturer with some pure hemoglobin solution, in contra-distinction to the American method of calibrating with the results of the Van Slyke oxygen-carrying capacity method whereby a higher calibration is obtained. Tests by Haden indicate that 100 per cent on the Sahli instrument corresponds to more than 14.0 grams of hemoglobin when calibrated with the results of the Van Slyke method now done in this country.

During the past year we have been plotting red cell counts from hemoglobin-values. In general, in our studies, they follow the same course.

It was noted, however, after the adjusted Newcomer had been in use for some months, that it was again giving readings which we had reason to believe were not true to fact. This evidence of instability of the Newcomer instrument seemed sufficient grounds for our coming eventually to consider the Sahli double-standard as the preferred apparatus.

SUMMARY

1. Experimental work demonstrated that there was wide discrepancy in the readings obtained by the several forms of apparatus commonly employed for the clinical determination of the hemoglobin-content of the blood.

2. The Van Slyke and the Wong methods are accurate and reliable, but they are too complicated for clinical use.

3. None of the simpler methods furnished correct readings until correction of the metering scales had been effected.

4. As a result of this study, the manufacturers now issue instruments with corrected standards.

5. It is a matter of choice whether the findings are reported in terms of percentage of normal, or in grams per 100 c.c.

6. Our findings as to the normal hemoglobin-content of the blood agree with those of German workers, 14.0 gm. Consequently, they are below those of Haden (15.6), and considerably below those of Williamson (16.92).

CONCLUSION

1. Because of its accuracy and stability, the new Sahli double-standard hemoglobinometer is the instrument of choice for clinical purposes.

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A COMPARATIVE EVALUATION OF THE RESULTS OF THE STANDARD KAHN PRECIPITATION PROCEDURE WITH THOSE YIELDED BY THE LAST TWO TUBES*

A Statistical Study†

By B. S. Levine,‡ Ph.D., Chicago, Ill.

THE object of this presentation is to compare the averages and the final values yielded by the standard Kahn precipitation procedure with those obtained from an evaluation of the results of the last two tubes. It was hoped that such a comparison would indicate whether or not the use of the last two tubes might be equally as reliable as the standard three-tube procedure. The practical significance of such a study in the case of a positive conclusion rests upon the considerable saving in time and material, resulting from the elimination of the first tube. The fundamental principles of the Kahn precipitation modification and its present clinical significance and applicability remain undisturbed.

The study is based upon 500 positive tests. The evaluations were made first by myself and then by an expert technician. The readings recorded represent a final agreement. Such a procedure of reading and recording the results of the precipitation test is in complete accord with the ideal requirements as set forth by the author of the test.[1]

The statistical study was carried out in groups as follows:

Group (a): Three-tube tests— 4.4.4; average—4.
(b): Three-tube tests— 3.3.3; average—3.
(c): Three-tube tests— 2.2.2; average—2.
(d): Three-tube tests— 1.1.1; average—1.
(e): Three-tube average—3⅔.
(f): Three-tube average—3⅓.
(g): Three-tube average—3, from readings other than 3.3.3.
(h): Three-tube average—2⅔.
(i): Three-tube average—2⅓.
(j): Three-tube average—2, from readings other than 2.2.2.
(k): Three-tube average—1⅔.
(l): Three-tube average—1⅓.
(m): Three-tube average—1, from readings other than 1.1.1.
(n): Three-tube average—⅔.
(o): Three-tube average—⅓.

The two-tube averages were as follows: 4, 3½, 3, 2½, 2, 1½, 1, ½.

To simplify the interpretation of the results in cases where the averages were represented by a compound fraction the usual arithmetic rule was adopted, namely: any fraction equaling or exceeding ½ was considered as

*Received for publication, February 15, 1931.
†Preliminary communication.
‡Director, Public Health Institute, Chicago, Ill.

equivalent to 1, while any fraction below ½ was discarded. This is also in accord with the system of interpretation of the precipitation results used by Kahn.[2]

SUMMARY OF THE RESULTS

Tests in complete agreement by the two separate interpretation procedures	423, or 84.6 per cent
Tests with final values higher by the two-tube interpretation:	
Three-tube value—3; Two-tube value—4	27 tests
Three-tube value—2; Two-tube value—4	1 test
Three-tube value—2; Two-tube value—3	22 tests
Three-tube value—1; Two-tube value—2	20 tests
Total	70 tests or 14 per cent
Tests with final values lower by the two-tube interpretation	
Three-tube value—4; Two-tube value—3	7 or 1.4 per cent
Total	500 or 100 per cent

No cases reacting definitely positively by the three-tube procedure are missed when only the last two tubes are used in the interpretation. Four hundred and twenty-three, or 84.6 per cent of the tests proved to have final values identical by the two methods of evaluation; 70 tests, or 14.0 per cent proved to have a higher, and only 7 tests, or 1.4 per cent, a lower final value by the two-tube interpretation. It is of considerable significance that only in one test, or 0.2 per cent, did the final two-tube value differ from the three-tube value by two, in all other cases the difference being equivalent to one.

Considering the general arbitrary character of the methods of serologic interpretation the difference between the results of the two- and three-tube evaluation of the precipitation results is, practically speaking, negligible.

In order to correlate his reaction with the nomenclature of the League of Nations Health Committee, Kahn suggests that the 4-plus, 3-plus, and 2-plus precipitation values be designated as *plus* (positive), and that the 1-plus and plus-minus values be designated as *plus-minus* (doubtful).[3] If this suggestion of the author of the test be accepted and the results of this study be grouped accordingly, then the difference between the two methods of reading and interpreting results becomes further reduced, as follows:

League of Nations Designation—*Plus* (positive):		
Three-tube values 4-plus, 3-plus, and 2-plus:	385	or 77.0 per cent
Two-tube values 4-plus, 3-plus, and 2-plus:	404	or 80.8 per cent
Difference (in favor of the two-tube test)	19	or 3.8 per cent
League of Nations Health Committee Designation—*Plus-minus* (doubtful):		
Three-tube tests	115	or 23.0 per cent
Two-tube tests	92	or 18.4 per cent
Difference	23	or 4.6 per cent

If the elimination or reduction in the number of doubtful reaction-designations is considered advantageous, then the difference of the 4.6 per cent in the above group of results becomes an item in favor of the two-tube method of interpretation.

League of Nations Health Committee Designation—*Negative:*	
Three-tube tests	none
Two-tube tests	4 or 0.8 per cent

According to this comparison the difference between the results of the interpretation of the three- and two-tube final values of the Kahn precipitation modification becomes negligible indeed. The elimination of the first tube in the performance of the Kahn test is an economical and time-saving procedure which in no way affects the principles of the test per se.

The complete report of this study will appear in a forthcoming issue of *The American Journal of Syphilis.*

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TREATMENT OF ACCIDENTAL PERIVASCULAR INJECTIONS OF ARSPHENAMINE OR NEOARSPHENAMINE*

By Theodore K. Lawless, M.D.,† Chicago, Ill.

PERIVASCULAR infiltrations may be due to various causes: lack of skill in administration; a highly sensitive nervous system, either in the patient or doctor; veins poor in size or accessibility; needles lacking in the necessary essentials—proper angle and type of point, length of bevel and sharpness; transfixion of the vein so that a portion of the solution is injected intravenously and the rest goes into the surrounding tissues. The latter accident carries with it a false sense of security, because on withdrawing the plunger there is a back flow of blood, giving the impression that the needle is entirely within the lumen of the vein.

The reaction of the tissues to infiltration with these irritating solutions is well known. The pain is sharp, lancinating and nerve wracking. The doughy edema of the tumor-like mass is sharply outlined by the intensely reddened skin which later demarcates itself into two zones—an inner deep purplish dusky hue, an outer collar of deep red. The secondary reactions are marked fibrosis and irritative responses on the part of the involved muscular tissues with functional disturbances due to marked contractures. Should sufficient of the solution be injected into the tissues a focal necrosis occurs with sinus formation from which a thin serosanguinous fluid exudes. This later becomes thick and gelatinous, with particles of tissue débris (Fig. 1). This painful tumefaction persists for weeks, even months.

*From the Department of Dermatology and Syphilology, Northwestern University Medical School.
Received for publication, July 14, 1930.
†Elizabeth J. Ward, Research Fellow.

What can be done in these unfortunate cases? The economic factor is one of considerable importance. Prophylactic and preventive technic are beautiful words and advice, but we are always face to face with the seemingly uncontrollable human factors. For immediate treatment we can make use of hot applications or cold compresses of magnesium sulphate solution supplemented by ice-bags. Dennie[1] has suggested the advisability of infiltrating the involved area with 2 c.c. of a 10 per cent solution of sodium thiosulphate followed by hot applications of 5 per cent solution of the same drug. In actual experience 2 c.c. injected blindly will never retrace the paths of the arsenical solution. In unresponsive cases he recommends multiple stab punctures followed by hot applications. In the regions usually involved important vascular and neurogenic units pass. Extreme caution is advisable. The tissues have already been insulted. Do not blindly incise them. Dietel[2] recommends the injection of sterile saline solution into the site of the infiltration, by so doing hoping to dilute the injected arsphenamine solution and

Fig. 1.—Maximum extension of arm before treatment.

hasten absorption. But due to muscular spasm and the insufficiency of leucocytic invasion the absorptive mechanism is hors de combat. We cannot expect too much from the lame. Kolmer[1] says that "if large amounts of solution have been injected and especially a syringeful of a concentrated solution of neoarsphenamine, I believe it is well to incise the area as soon as possible under local or general anesthesia and gently massage out as much of the solution as possible. Hot applications should now be applied." But why incite the surgical instinct more? The great problem today in that field is curtailment. To what depth and length will you incise? How can one identify the arsenical washed tissues immediately? Be careful of the capsule of the elbow joint and do not forget the chances for secondary infection of surgical wounds.

It has seemed to me that our problem here is a chemical one, and relief must be sought along this same line. So we turn to our old friend, sodium thiosulphate, a well-known enemy of arsenic. The following mode of application is offered as productive of results.

One gram of sodium thiosulphate is dissolved in 15 c.c. of sterile distilled water and placed in a suitable syringe. Place the arm band of a blood-pressure apparatus, folded if necessary, as high above the infiltration as possible. Inflate until both venous and arterial circulations have been stopped. Insert a needle with syringe attached into a vein as far below the infiltration as possible. Withdraw 10 to 20 c.c. of blood. Detach the blood-containing syringe and attach the one containing the sodium thiosulphate solution. Slowly inject 10 c.c. of this solution. Then gradually release the constrictor, at the same time injecting the remaining 5 c.c. Remove needle and apply pressure to the point of insertion.

The process here consists of the following elements: (a) we have used a chemical antidote, (b) nature's own channels have been used for forcing the solution into the tissue spaces, and (c) arterial blood and pressure, due to the application and release of the constrictor, have been employed to force the arsenic-laden tissue juices into the proper excretory pathways.

Fig. 2.—After second injection.

Fig. 1 illustrates a case of arsenical infiltration with sinus formation. The Abelin Test applied to the blood and to the tissue juice forced through the sinus gave positive reactions for the presence of arsphenamine.

Abelin's Test:[3] Remove 5 to 8 c.c. of blood from one of the arm veins. Use an absolutely dry syringe and test tube. Permit the blood to stand or centrifuge until serum and clot have separated. Use only clear serum. Remove 1 or 2 c.c. of the clear serum. Place into a dry test tube. Cool under running tap water. Add 2 drops of dilute hydrochloric acid. Shake well. Now slowly add 3 drops of 0.5 per cent sodium nitrite solution. Shake well.

In a second test tube dissolve 0.3 gm. of resorcin pur. or alpha-naphthol (Kahlbaum) in 4-5 c.c. of 30 per cent sodium hydroxide.

To this alkaline solution of resorcin or alpha-naphthol add very carefully and by drops the acidified serum.

In the presence of arsphenamine a beautiful red colored ring is formed at the junction of the two solutions.

From April 29, 1929, to June 10, 1929, this patient received in our Clinic a total of 4.35 gm. of neoarsphenamine intravenously divided into eight doses given at weekly intervals. On July 8 and 15, 1929, he also received a total of

four grains of mercury salicylate intramuscularly. He received no other antisyphilitic treatment until October 8, 1929, when he consulted another physician, "who had some genuine old German 606." Unfortunately, during the first injection a large amount of the solution, judging from the results, was introduced outside the vein. The reaction was intense. The lower two-thirds of the upper arm was swollen, red and hard. Because hot applications and treatments with some kind of therapeutic lamp were not productive of results the patient came back to us and on April 23, 1930, received his first treatment of sodium thiosulphate under the above described pressure method. Following this injection the pain was relieved and 70 per cent of the spasticity disappeared. On April 25 a second injection was given. On April 30, a third; then at weekly intervals for three injections. Following the fourth injection the arm functioned normally, and the sinus was closed. There remains a very slight amount of superficial infiltration.

CONCLUSIONS

1. In a patient with a tumor caused by perivascular infiltration of arsphenamine the blood as well as the tumor juice gave positive tests for arsphenamized arsenic seven months later.

2. The injection of sodium thiosulphate under pressure gave immediate relief of the pain.

3. The muscular spasm was equally influenced.

4. Necrosing arsphenamine lesions yield in a surprisingly short time when compared with results obtained by other methods.

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4201 South Parkway.

FIXATION OF THE BRAIN FOR ANATOMIC EXAMINATION*

By Frank M. Kramer, New York, N. Y.

THE most frequent method used in fixing the whole brain for anatomic sectioning consists in suspending it in a 10 per cent solution of formalin. Satisfactory fixation by this method requires from three to five weeks. The delay in completing autopsy protocols under such circumstances is obvious.

An "injection" method for rapid fixation of the whole brain preparatory to sectioning is here described.

In order to insure success with this method, meticulous care must be exercised in the removal of the organ from the cranial cavity. Tearing of the pia meninges and laceration of the cerebral tissue from careless use of the saw in opening the calvarium militate seriously against the probability of obtaining a satisfactory result with the technical method under discussion. It is important to sever the basilar vessels close to the base of the skull, thus leaving enough of each vessel connected with the brain to allow for the insertion of a cannula into the necessary lumen or lumina and for their ligation. This is especially the case with regard to the vertebral and internal carotid arteries. It is also essential to deliver the small brain with great care, and the chances of injuring this portion are minimized by first cutting through the tentorium its full length on both sides.

After removal, the brain is placed in a solution of sodium chloride of such strength and specific gravity that the specimen will float therein. The brain must never be allowed to rest on the bottom of the vessel containing the salt solution, since its own weight is sufficient to flatten the cerebral surfaces in such measure as to interfere with the proper injection.

A small cannula is now introduced into the lumen of one of the carotid arteries and ligated in position. The stumps of the other vessels are either ligated or clamped with hemostatic forceps to prevent leakage. In general, the adequate collateral circulation (which is secured by the arrangement of the basilar arteries) renders it possible to inject the whole brain through the one carotid, unless there be some pathologic occlusion of a member of the Circle of Willis or at some other point in the larger cerebral vessels. In such case it may be necessary to employ both carotids, or to direct the cannula into the lumen of the artery supplying that portion of the brain which fails to inject in the ordinary manner. The fixing fluid consists of a 10 per cent solution of formalin. This is to be slowly injected (after previous expulsion of all air from the injecting apparatus) until the brain presents a moderately firm distension. Too rapid and excessive injection must be avoided. Too great pressure exerted during the process may rupture one or more vessels with consequent distortion and irregular or incomplete fixation.

*From the New York State Psychiatric Institute and Hospital, New York City.
Received for publication, March 24, 1931.

Unfortunately, difficulty is often encountered in the attempt to secure satisfactory injection of the cerebellum. In such instances this portion of the brain must be injected separately and additionally through the inferior cerebellar arteries.

After the injection process has been completed, the brain is to be suspended in a 10 per cent solution of formalin and allowed to "set." From six to twelve hours will suffice for complete fixation and permit gross sectioning. Sections thus made retain their conformation. Blocks for histologic examination may be removed at this time, and, since they are already sufficiently fixed, their dehydration may be begun at once.

For the purpose of anatomic demonstration, a colored emulsion of starch may be injected in conjunction with the formalin solution, and painstaking attention to the details of the technic will secure results very definitely superior to those obtainable by injections of similar emulsions into the axillary, femoral, or other vessels of the body before removal of the brain.

Elaborate apparatus is not required. Excellent results may be obtained with a large Luer syringe and a cannula improvised from such a needle as is ordinarily used for the transfusion of physiologic salt solution.

In certain cases of cerebral thrombosis, abscess, hemorrhage and extensive softening, and in other conditions involving destruction or distortion of the brain, this method may fail. On the other hand, surprisingly good results are not infrequently obtained even when fairly extensive pulpification has taken place. Thus, in one case, the method was successfully employed in the preparation of a brain of which a good portion of the temporo-sphenoidal lobe had been pulpified by a gunshot wound. The method cannot be used, of course, on cases where a pathologic investigation of the brain demands the use of several different fixing agents.

The advantage which is to be most emphasized in connection with the above-described procedure consists in the possibility of sectioning and of macroscopic examination of the brain within a few hours after autopsy and while the findings in other structures and viscera are still vivid in the mind of the pathologist.

722 West One Hundred Sixty-Eighth Street.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Diphtheria: A Modified Loeffler's Blood Serum Medium, etc., Medalia, L. S., Bailey, K., and Atwood, C. J. Bact. **21**: 119, 1931.

The formula recommended is:

Beef extract broth P_H 7.2, 1 part, containing 1 per cent glucose, 5 per cent glycerol, pig serum P_H 6.8, 3 parts; to which was added 4.5 per cent N/1 sodium hydroxide, the final P_H being 7.6 (determined colorimetrically with Brom-thymol blue as an indicator).

The medium is semitransparent and stated to be almost selective for B. diphtheriae and streptococci.

Intestinal Protozoa, Incidence of Human, Andrews, J., and Paulson, M. Am. J. Med. Sc. **181**: 102, 1931.

A survey for human intestinal protozoa with especial reference to Endameba histolytica among adults residing in their natural environment in a large American city of the temperate zone (Baltimore) who appeared at the out-patient department for relief from gastrointestinal symptomatology. This study is distinctive in that the material has come from those residing in their homes, while practically all similar investigations have been among institutional and military classes, rural groups and from highly concentrated zones of population where fecal contamination was common. While the total numbers of cases examined is not great, the correspondence between the results in two series herein reported is so remarkably close, that the conclusion is justified that the incidence of the various protozoa infecting the human intestinal tract in this locality is not as great as has been frequently expressed for the temperate zone; and further, that the following incidence among this group of city dwellers is probably more nearly representative of the incidence of intestinal protozoa in residents of the temperate zone as a whole, inasmuch as most of the population of the temperate zone is concentrated now within large cities.

In 522 cases studied, the total incidence of human protozoa infesting the intestinal tract was 10.9 per cent. By species: Endameba histolytica, 0.2 per cent; Endameba coli, 4.2 per cent; Endolimax nana, 2.5 per cent; Iodameba Williamsi, 1.3 per cent; Trichomonas hominis, 2.1 per cent; Chilomastix mesnili, 1.3 per cent; Giardia lablia, 2.7 per cent; Embadomonas intestinalis, 0.2 per cent.

Blood Platelets, Response of to External Stimuli, Steiner, P. E., and Gunn, F. D. Arch. Path. **11**: 241, 1931.

Ninety enumerations of the blood platelets, leucocytes and erythrocytes in the blood of fourteen normal rabbits were made. The average normal platelet counts in this group of animals were somewhat higher than those reported by other investigators.

The effect of ultraviolet light irradiation on blood platelets as reported by previous investigators was corroborated.

Iodine applied to the backs of rabbits caused thrombocytosis, beginning in from two to five days, reaching a maximum by the sixth day, and slowly receding to a normal level in from two to three weeks. Marked secondary anemia sometimes occurred within twenty-four hours, with a return of the erythrocytes to normal in from two to three weeks.

Coal tar applied to the back was followed by either thrombocytosis or thrombopenia. The numbers of the other blood elements were not appreciably changed.

The similarity between the effects on the platelet level of ultraviolet light and of other substances producing dermatitis suggests that the effects may be due to tissue injury and to the absorption of breakdown products that stimulate the formation of platelets.

Anemia, Pernicious, The Hemoglobin Content Volume and Thickness of the Red Blood Corpuscles in Pernicious Anemia and Sprue and the Changes Associated With Liver Therapy, Wintrobe, M. M. Am. J. Med. Sc. **181**: 707: 1931.

A series of 100 blood examinations has been carried out in 16 cases of pernicious anemia and 8 cases of sprue, of all degrees of severity and at varying intervals during remissions induced by liver therapy. Mean corpuscular volume, corpuscular hemoglobin, and corpuscular concentration were determined at each examination and in 32 instances cell diameter and mean corpuscular thickness were measured as well. As a result of these determinations the following conclusions seem justified.

1. The mean volume (mean corpuscular volume) and the hemoglobin content (mean corpuscular hemoglobin) of the red corpuscles in pernicious anemia and sprue are significantly greater than normal during the stages of relapse of these diseases. The deviations of these values from the normal are of such magnitude and occur so constantly that they afford a simple and readily available aid in diagnosis and are useful in observing the alterations in the size and hemoglobin content of the red corpuscle which are associated with remission and relapse in these diseases.

2. Increases in mean corpuscular volume are of much greater magnitude than corresponding increases in average cell diameter and therefore the determination of mean corpuscular volume offers the more useful means of estimating variations in the mean size of the red cells. Even during the period of severe anemia at a time when numerous small and distorted cells are to be found in the blood, corpuscular volume values which were significantly greater than normal were constantly found. The greater value of mean corpuscular volume as compared with mean cell diameter determinations is explained by the fact that increases in the size of red corpuscles in all dimensions are observed when the mean corpuscular volume is calculated.

3. Both mean corpuscular volume and mean corpuscular hemoglobin are highly correlated with the red cell count in pernicious anemia and sprue. The correlation is linear and the sign of correlation is negative; that is, high values for these constants are found when the cell count is low and lower values are observed as the cell count rises. The correlation between mean corpuscular volume and the cell count is less marked in the severe stages of anemia than when the anemia is less marked (red cell count above 2 million) probably because of the presence of large numbers of small and distorted cells in the blood stream at such a time.

4. The upper limit of normal mean corpuscular volume has been arbitrarily fixed at 95 cu. micron. Higher values than this have rarely been found in conditions other than pernicious anemia or sprue.

5. In marked contrast to the values for corpuscular volume and corpuscular hemoglobin the concentration of hemoglobin in the red corpuscles showed no correlation whatever with the red cell count. Neither was there noteworthy correlation between corpuscular concentration and either corpuscular volume or corpuscular hemoglobin. The variations in corpuscular concentration were small in extent and of little greater magnitude than has been observed in normal individuals.

6. Abnormally high values for corpuscular concentration were not found at any time in the cases of pernicious anemia and sprue and no support has been found for the frequently repeated statement that the red corpuscles are supersaturated with hemoglobin in these diseases.

7. Low values for corpuscular concentration were found only at the time of unusually active blood regeneration. This significance of this observation both as regards the pigment metabolism and the treatment of pernicious anemia and sprue, is briefly discussed.

8. Mean corpuscular volume and mean corpuscular hemoglobin were highly correlated with each other. It appears that a significant change in the blood in pernicious anemia and sprue is an increase in the mean volume and hemoglobin content of the red corpuscles. The increases in volume and in hemoglobin content tend, on the whole, to be parallel, with the result that the concentration of hemoglobin in the corpuscles is not significantly altered except under unusual circumstances.

Drawings, Pen and Ink, from Photographs, Maylor, E. Science 73: 15, 1931.

The method is as follows:

In general, the procedure is to photograph the material, make a print on a good grade of paper and draw over it with India ink. All stippling or other shading may be done directly on the photograph. The print is then placed in solutions which bleach away the photographic image and leave the ink tracing standing out on white background. If the original photograph is so small that details cannot be drawn in easily, it may be enlarged several times, treated as above and the drawing reduced in reproduction.

The process of removing the photographic image is accomplished by two solutions. The first dissolves away the photographic image and the second bleaches the paper.

Solution 1.	
Potassium iodide	15 gm.
Iodine	5 gm.
Water	500 c.c.
Solution 2.	
Hypo crystals	100 gm.
Water	450 c.c.

When the ink on the drawing is thoroughly dry, quickly immerse the print in solution 1. Rock the tray immediately so that the solution covers the print rapidly and evenly. The photographic image will disappear almost at once, and at the same time the print will become brown from the iodine. As soon as all traces of the photograph have disappeared, remove the print and wash gently in water in order to remove the excess iodine. Then place in solution 2. Here the brown color is completely lost and the print becomes quite white in about five minutes. Transfer to water and wash thoroughly to remove the "hypo." Dry the print by placing it in a horizontal position on blotting paper. It will curl as it drys, but later it can be flattened by dampening the back and placing in a press. Throughout the entire process care should be taken that nothing touches the surface on which the drawing has been made, for the ink smears very easily while wet. The iodine solution may be used repeatedly until it becomes too weak, when it may again be brought to strength by adding more iodine. The "hypo" solution may likewise be used many times.

After the chemical treatment no traces of the photograph remain, and the drawing stands out on a white background without any staining or blurring of the print. If desired, pencil or even charcoal may be used in place of the ink.

Tissue: Frozen Sections Prepared by Use of "Dry Ice," Lindsay, J. W., Rice, E. C., Selinger, M. A. J. A. M. A. 96: 773, 1931.

The special equipment necessary and which may be attached to any microtome consists of an "ice" chamber with a freezing plate, and a plunger with which the "ice" may be brought into contact with or released from the plate. The freezing material is the commercial "dry ice" readily obtained from ice cream packers at a small cost. A block of this substance weighing 5 pounds may be kept in the cardboard container in which it is delivered and at ordinary room temperature for forty-eight hours. By the use of thoroughly insulated containers it is hoped to extend the life of a supply beyond this time. However, with this time limit, it is seen that many possibilities for preparing sections by the freezing method, at long distances from the hospital or even from the city, are offered. No other equipment than the fixing and staining materials, a small block of "dry ice" and a small portable microtome are necessary. The entire apparatus and materials may be carried in a small case.

Pertussis: Early Bacteriological Diagnosis: Sauer, L. W. Science 73: 125, 1931.

In the December, 1930 issue of this Journal (p. 321) the technic evolved by Sauer and Hambrecht for the "cough plate" diagnosis of whooping cough was described.

Sauer now adds the following details as of importance:

1. The medium should be sterile, bright red and the surface should not be dry.

2. A series of Petri dishes should be inoculated with B. pertussis and the small, discrete, round, elevated, shiny gray colonies should be studied from day to day. On the third to fifth day the colony approximates 1 mm. in diameter and is surrounded by a zone which appears translucent in transmitted light and darkened in reflected light.

3. Duplicate plates should then be exposed to early cases of known pertussis. Overgrowth by mouth saprophytes can in part be avoided if the child drinks water just before the plates are exposed. An uncovered plate is held vertically, a few inches from the open mouth at the moment of expulsive coughs from the deeper bronchi. The plate should be incubated at 37° C. within a few hours, and be examined daily for four or five days. Rapidly growing saprophytes should be cut out with sterile platinum wire. The characteristic, zoned colonies usually appear on the third to fifth day. A hand lens used in bright light is helpful in finding the raised, circular colonies in thickly seeded plates. Poorly exposed plates should not be incubated. After mastery of the technic, aluminum boxes (4 cm. × 1.5 cm.) may be used. They require less medium, can conveniently be carried, and dry out more slowly (broad rubber band over seam).

Pertussis organisms are minute, oval, gram-negative bacilli which stain feebly. Polar staining may be present. If the cough has already persisted for several weeks, plates exposed to the other, susceptible children of the family will more likely be positive. A negative plate does not exclude pertussis, and a second plate may be positive. If the cough has persisted too long, or if it is not whooping cough, pertussis bacilli will not be found.

Hyperthermy, Production of. Spinelli, A. Biochem. terap. sper. **17**: 397, 1930.

The substance extracted from yeast by the method described below, when injected intravenously (0.7-0.8 c.c.) has been found to produce fever lasting five or six hours and is recommended, therefore, for experimental purposes.

Mix 500 c.c. of water, 250 gm. of yeast, and 10 c.c. of 5 per cent sodium hydroxide and autolyze at room temperature for fifteen to twenty-five days. The filtrate is used for injection.

WASSERMANN TEST: A More Sensitive Antigen, Eagle, H. J. Exper. Med. **53**: 605, 1931.

One hundred gm. of dry powdered beef heart muscle are extracted with 500 c.c. ether for fifteen minutes at 37° C. with shaking. After filtration *with suction*, the ether filtrate is discarded. The powder is then dried and extracted for from three to five days with 500 c.c. of 95 per cent alcohol, with intermittent shaking. The mixture is filtered, and the moist powder washed on the filter paper with two portions of alcohol, each 100 c.c. The alcoholic filtrate and washings are combined and evaporated on the steam bath down to 250 to 300 c.c. Cholesterol (0.8 per cent), and sitosterol (0.6 per cent) are then added, and dissolved at 65° to 75° C. The excess sensitizer which crystallizes out upon cooling is dissolved just before using by immersing the antigen in a 56° C. bath for a few minutes. It is diluted by pouring the saline rapidly into the antigen. A 1:40 dilution is recommended for use in water-bath fixation (one-half hour at 37° C.), as well as for the short ice box fixation (8°, four hours) and a 1:120 dilution for use in the overnight ice box method (sixteen to twenty-four hours at 8° C.), as being well beyond its anticomplementary range; the anticomplementary quantities being 1:5 and 1:25 respectively.

UROBILINOGEN: Average Daily Elimination in Health and Disease, Especially in Pernicious Anemia: Standardization of Method Based on Mesobilirubinogen, Watson, C. J. Arch. Int. Med. **47**: 698, 1931.

Collection of Feces: If the patient is having regular defecation of formed stools daily at relatively the same time, it is sufficient to save all stools for four days, counting from an hour at which one was discarded. The last stool is collected at relatively the

same hour four days later. Specimens for the first and second forty-eight hours are collected separately and analyzed separately at the end of two- and four-day intervals. In this way the output of urobilinogen may be followed over some period by the daily average for consecutive two-day periods, or the daily average for a single four-day period may suffice. Specimens are most conveniently kept in the ordinary cylindric waxed cardboard cartons of liter capacity. These will usually contain all of the forty-eight-hour stool, unless enemas have been necessary. They should be kept in a cool place, with exclusion of light, during the period prior to analysis.

Measurement and Mixture of Stool Specimens: The entire forty-eight-hour specimen must be weighed prior to analysis. An empty container of the same size as that containing the specimen is placed on the balance pan opposite the specimen. In this way the stool may be weighed directly. The forty-eight-hour stool specimen must be mixed so that all parts are relatively equal. In the case of stools having at least a moderate degree of consistency, mixture may be made in the collection container directly. The iron rod of a ring stand is useful for this purpose, as it is easily cleaned. Stools that are liquid or partly so must be thoroughly homogenized in a large mortar. If the specimen is partly solid and partly liquid, mixture is best accomplished by first pouring the liquid portion into a beaker, transferring the solid portion to the mortar where it is ground up with the pestle, and then adding the liquid from time to time until all is thoroughly mixed.

Method of Quantitation: The following materials are required: 1 liter mortar and pestle; one small mortar and pestle; one 250 c.c. evaporating dish; one suction flask of 250 c.c. capacity equipped with a 12-inch length of heavy walled rubber tubing fitted on the suction arm; a few plain stiff clamps or hemostatic forceps (strong pinchcocks may be used); several 5 c.c. pipettes graduated in 1 c.c., and 1 c.c. pipettes graduated in 0.1 c.c.; a spectroscope (small hand size is sufficient); a van Slyke type of separatory funnel capable of holding 250 c.c.; a few ordinary 15 to 20 c.c. test tubes and a few 50 or 100 c.c. graduated test tubes or cylinders, and a colorimeter, preferably of the Duboscq type, although any reasonably accurate colorimeter may be used. In the present series of estimations, a Klett biocolorimeter was employed. If more than one determination is to be made, it will be necessary to have two or three of the suction flasks.

The following reagents are required: Ehrlich's reagent, composed of 10 gm. of paradimethylaminobenzaldehyde, 75 c.c. of concentrated hydrochloric acid and 75 c.c. of distilled water; crystalline ferrous sulphate ($FeSo_4$ 12 H_2O); 10 per cent sodium hydroxide; 5 per cent sodium amalgam; 20 per cent acetic acid; pure ether (diethyl); saturated ethereal solution of paradimethylaminobenzaldehyde (should be light yellow, as definite redness or darkness indicates impurity); concentrated hydrochloric acid in a dropping bottle; a saturated solution of sodium acetate (aqueous); a saturated solution of sodium carbonate (aqueous); 0.05 per cent alcoholic solution of phenolphthalein (50 mg. of pure phenolphthalein in 100 c.c. of 95 per cent alcohol kept in a glass stoppered bottle will suffice for a large number of determinations).

Procedure for Analysis: Weigh out 5 gm. of the mixed stool on filter paper, or if not sufficiently dry, in an evaporating dish. Transfer to a small mortar. The ordinary cheap wooden tongue depresser is very useful in weighing and transferring. Add a small amount of water from a measured portion of 95 c.c. Grind thoroughly with a pestle; it is important that the particles of feces be finely ground. Adler has observed that small fecal particles adsorb urobilin and hinder extraction. Add the remainder of the water and again mix completely. Let this mixture stand a moment or two while measuring out 50 c.c. of 10 per cent sodium hydroxide. Decant the supernatant portion from the mortar into a small flask (Florence, preferably). Again grind up the remaining precipitate and fluid, then add the 50 c.c. of sodium hydroxide and add all of this to the first portion in the flask. Shake vigorously for a few moments. Pulverize 10 gm. of ferrous sulphate on the filter paper on which it was weighed and place in the 250 c.c. suction flask, the side arm of which is fitted with heavy walled rubber tubing. Add 46 c.c. of distilled water and shake until the majority has gone into solution. Now add to this in small portions and with vigorous shaking all of the fecal emulsion from the other flask. Cork tightly and evacuate with suction. When vacuum is considerable, clamp the rubber tube with forceps or stiff clamp. Set aside in the

dark for one hour. At the end of this period, filter from 3 to 4 c.c. into an ordinary test tube. Examine this with a hand spectroscope for the presence of a definite absorption band of alkaline urobilin (495-515). Rarely more than a faint urobilin band is seen, and in this event further reduction is necessary. To approximately 15 c.c. of the filtrate in a 50 c.c. graduated centrifuge tube, add from 1 to 2 gm. of 5 per cent sodium amalgam. Cork tightly and set aside in the dark for ten minutes. A second period of ten minutes may be necessary for disappearance of the urobilin band. If sodium amalgam is not at hand, the following method may be substituted: weigh out another 5 gm. of the mixed stool specimen and proceed exactly as before, except use double the amount of both the 20 per cent ferrous sulphate and the 10 per cent sodium hydroxide (100 c.c.); this makes the value of the primary dilution 60, while it is ordinarily 40.

To a few cubic centimeters of the urobilin-free filtrate (as examined spectroscopically) in a test tube, add Ehrlich's reagent from a dropping bottle, drop by drop, until cloudiness has disappeared. The amount of red which develops immediately determines roughly how much filtrate is to be used in the quantitative determination. The following is a useful, but not necessarily absolute, list of the degrees of color and corresponding amounts of filtrate to be used: (1) deep red with a slight bluish tinge, 1 c.c.; (2) light red, 2 c.c.; (3) pink, 5 c.c.; (4) barely discernible pink, 10 c.c.; (5) no color and absent urobilinogen-aldehyde absorption band (550-570), from 25 to 50 c.c. (If a new specimen, 50 c.c. should be employed, but if color has recently been present in previous specimens, 25 c.c. is probably enough).

In a series of determinations made every other day on specimens from the same patient, after the first day it is usually sufficient to use the same amount of filtrate each time.

Place the amount of filtrate chosen in a separatory funnel and acidify with an excess of 20 per cent acetic acid (0.4 c.c. per cubic centimeter of filtrate constitutes a definite excess). At once add approximately 40 c.c. of pure ether (diethyl). The amount need not be exactly measured. Shake vigorously for from two to three minutes. (When larger amounts of filtrates are used, the shaking must be relatively more gentle; from 75 to 100 inversions will suffice.) Allow the aqueous fraction to separate, then drain it off and discard. Wash the ether once with a small portion of distilled water, and discard the latter. From a 5 c.c. pipette add 3 c.c. of a saturated ethereal solution of paradimethylaminobenzaldehyde. Add 10 drops of concentrated hydrochloric acid from a dropping bottle. Shake vigorously for from one to two minutes. Add a few cubic centimeters of distilled water and shake briefly. Add 3 c.c. of a saturated aqueous solution of sodium acetate and again shake. Separate off the colored solution into a 50 c.c. graduated centrifuge tube or cylinder. Place this in a glass or rack and cover temporarily with a cloth or towel. Repeat the following extraction, using only 5 drops of hydrochloric acid, a smaller amount of water and 2 c.c. of the sodium acetate solution. More of the aldehyde need not be added. If not too much filtrate is chosen in the first instance, three such extractions will suffice. Sometimes when the amount of urobilinogen is large, four or five extractions will be necessary. The last extraction should yield only a faint color. When all of the colored aqueous portions have been collected in the same graduated tube, this collection is made up to a volume which is a multiple of the original amount of filtrate taken. If the color is pale, dilution should be only to the next cubic centimeter. This solution is thoroughly mixed, and a portion of it is placed in one colorimeter cup. Some of the standard phenolphthalein solution is placed in the other cup. This is composed as follows (according to Terwen): 1 c.c. of 0.05 per cent alcoholic solution of phenolphthalein, and 5 c.c. of saturated aqueous solution of sodium carbonate. Make up to volume with distilled water in 100 c.c. volumetric flask. Prepare fresh the same day.

As subsequently shown in detail, this standard solution has a color equivalent to that of 0.4 mg. of urobilinogen condensed with paradimethylaminobenzaldehyde and hydrochloric acid in 100 c.c. Calculations are then as follows: D_1 (usually 40) $\times$ D_2 = $\frac{\text{volume of final solution}}{\text{volume of filtrate used}} \times \frac{S}{R} \times 0.4$ = mg. of urobilinogen in 100 gm. of stool. Multiply this by the weight of the entire stool specimen and divide by 100 to obtain the total amount of urobilinogen. At the end of the period of collection (usually four days), the amounts obtained are added together and the sum divided by the number of days. This final result is the average daily output in milligrams.

The foregoing extraction procedure should be carried out in subdued light, preferably artificial.

It is essential that no alkali should come in contact with the final colored solution, as the dyestuff is decomposed by alkali, with complete loss of the red color (Fischer).

Collection of Urine: Urine is collected for two forty-eight-hour periods in gallon bottles in which have been placed from 20 to 30 c.c. of a saturated alcoholic solution of salicylic acid, for preservation. (The salicylic acid has been shown by Terwen not to have any effect on the subsequent estimation). During collection, these bottles should be kept in a cool, dark place.

The forty-eight-hour urine should be mixed and measured. If deeply colored or if bilirubinuria is suspected, bilirubin should be tested for, the Gmelin, Huppert-Cole or van den Bergh test being used. If bilirubin is found to be present in more than traces, preliminary treatment is necessary to eliminate it, as with the foregoing method a certain amount of color may be produced by the reduction of bilirubin alone, in the absence of urobilinogen. (Pure bilirubin was dissolved in 10 per cent sodium hydroxide; this was added to 20 per cent ferrous sulphate as in the foregoing procedure, and after standing in the dark one hour the filtrate was found to give a definite aldehyde reaction with the same extraction technic used for the stool filtrate. Terwen apparently did not consider that the bilirubin was reduced in any degree as he advises the use of petroleum ether in place of ether which, he states, does not extract bilirubin from the acidified filtrate.)

To free the urine of bilirubin, add to 200 c.c. of urine 100 c.c. of a saturated aqueous solution of calcium hydroxide with slight excess not in solution, and to which has been added a relatively small amount of 10 per cent sodium hydroxide. Shake these solutions together in a beaker and let stand for a short time. The bilirubin is precipitated as insoluble calcium bilirubinate. Then filter. The filtrate is used in place of bilirubin-free urine in other determinations and the added dilution of 1.5 is taken into account in the final calculation.

To 5 gm. of ferrous sulphate in the suction flask with rubber tubing on the suction arm, add 23 c.c. of distilled water. To this add 100 c.c. of the mixed bilirubin-free urine, or filtrate to be tested. Then add in small portions at a time and with constant shaking 25 c.c. 10 per cent sodium hydroxide. Evacuate, clamp, and set aside in dark for one hour. The procedure from now on is the same as in the case of the determination on the stool, except that it is necessary to use larger amounts of filtrate, especially with normal urines and bilirubin filtrates of urines containing small amounts of urobilin. Fifty c.c. of filtrate should be employed in such instances.

Normally, there is rarely more than a trace of urobilin present in the urine, and the amount of color obtained from 50 c.c. of filtrate is often so small that accurate comparison of the color is impossible. It is better in such an instance to report simply a trace, rather than a figure which is not liable to be accurate. In the present series more than 50 c.c. of filtrate have not been used.

When urine alone is used, the method of calculation is as follows:

$$D_1\ (1.5) \times D_2 \left(\frac{\text{Final volume}}{\text{Amount of filtrate}}\right) \times \frac{S}{R} \times 0.4$$

This multiplied by the number of cubic centimeters of urine in the entire specimen and divided by 100 gives the total number of milligrams present.

When calcium hydroxide has been used to precipitate bilirubin, the calculation is done as follows:

$$D_1\ (1.5) \times D\ (1.5) \times D_2 \left(\frac{\text{Final volume}}{\text{Amount of filtrate}}\right) \times \frac{S}{R} \times 0.4$$

BASOPHILIA: Punctate, Demonstration by Dark-Field Illumination, Nelson, W. T. Med. J. Australia **14**: 310, 1931.

Microscope: A binocular microscope with a mechanical stage was used.

Objective: A "Zeiss" apochromatic oil-immersion objective of numerical aperture "1.0" was found to be the best for the purpose. This objective is made especially for dark-

field work and is fitted with an integral iris diaphragm, so that the aperture may be reduced if necessary.

Oculare: Compensating eyepieces of ten magnifications were used.

Condenser: A "Siedentopf change-over condenser," made by Zeiss fulfilled all requirements. In this condenser provision is made for the observer, by moving a lever, to change from bright to dark-field conditions, or vice versa. A tinted ground glass, built into the condenser, prevents dazzle when the bright field is used with the arc lamp.

Lamp: An arc lamp, preferably clockwork driven, is required to give the best results with the "Siedentopf change-over condenser." A "Zeiss" arc lamp was used with the direct current. The lamp is fitted with an iris diaphragm, aiding one in rendering the dark field as black as possible.

METHODS AND RESULTS

When observing a stained blood film by means of the dark-field method, the leucocytes first attract notice because of the way in which they are lit up. The normal erythrocytes, on the other hand, appear structureless and their appearance resembles bright rings on the black ground. When, however, granules are present within the corpuscle, as in punctate basophilia, bright particles appear, scattered throughout the cell. Bright objects on a dark background are more readily seen than dark objects against a bright field. This is particularly noticeable when the objects in question, basophilic granules, are very tiny. The ordinary oil-immersion objective used in examining blood films under the usual bright field conditions has a short depth of focus, and, unless one is constantly refocussing, fine stippling, when present, is apt to be overlooked. By use of the dark-field one is not so liable to miss the tiny granules through inaccurate focussing.

Cells with diffuse polychromatophilia are often not easily seen. Under bright field conditions, in a film stained either by Leishman's or Giemsa's method, such cells have a bluish appearance, in contrast with the red color of the other erythrocytes. While it is difficult to see any granules in some of the polychromatophile cells, most cells of this type examined by means of the dark-field are observed to have a fine grade of stippling identical in appearance with that found in many cells stained by Sellers' method. Examination of these, by means of bright field illumination, merely reveals a deeper staining, and differentiation cannot be made with certainty. By means of the dark-field, they are easily recognized as finely stippled cells.

COMPLEMENT: A Method of Drying From the Frozen State, Craigie, J. Brit. J. Exper. Path. **12**: 2, 1931.

The present method takes advantage of the following facts:

1. Water may be frozen if rapid evaporation be brought about under suitable conditions.

2. Water may pass from the solid to the gaseous state without obviously passing through the aqueous phase.

3. Complement retains its activity best at a low temperature. The apparatus required is simple, only a vacuum pump (Geryk) and a desiccator of suitable design being necessary. The desiccator contains dry calcium chloride, a thin glass dish to hold the complement and a support for the latter. The support should be so designed that it virtually brings about thermal isolation of the complement and at the same time occludes as little space as possible. An X-shaped wire frame, the four ends of which are fixed into pieces of cork, forms a suitable support. The dimensions of the dish depend on the volume of complement to be dried, and should be such that the depth of fluid is at least one centimeter. There should be ample clearance between this dish and the sides of the desiccator and the calcium chloride.

The guinea pig serum is placed in this dish and the desiccator is luted down with rubber-vaseline compound (rubber solution [chemico] 1 part; vaseline 2 to 5 parts; heat until all solvent has evaporated). The desiccator is now evacuated. Slight bubbling will take place, and the temperature of the complement will drop rapidly until it suddenly freezes solid. Previous chilling of the complement will hasten freezing, but this is unnecessary, for freezing will occur in any case in approximately ten minutes. When the comple-

ment has become frozen the desiccator tap is closed and the apparatus is left at room temperature. No further supervision is necessary for the complement will remain frozen until dry because the evaporation of the water automatically keeps it several degrees below 0° C. Drying of the complement under such conditions is surprisingly rapid; six hours suffice to dry 50 c.c. of complement, but it is advisable to leave it drying overnight. The removal of the dried complement presents no difficulty, for it spontaneously detaches itself from the glass surface in the form of pink, readily pulverizable masses. Any dry serum forming a thin film firmly adherent to the glass should be discarded. The dried complement should be stored in vacuo over calcium chloride at a low temperature. The ratio of dry weight to original fluid volume will, of course, have been determined in order to ascertain the volume of distilled water required to reconstitute the complement from a given weight of the dried product. On an average 1 gm. of dried complement is equivalent to 16 c.c. of original complement. The dried complement dissolves in the required volume of distilled water very rapidly but it is advisable to allow the solution to stand for at least half an hour before ascertaining its hemolytic titer.

This method has the following advantages over the method of vacuum distillation of the water at 37° C.:

1. The complement is automatically kept below 0° C. until it is perfectly dry.

2. The frozen complement cannot froth, and therefore the globulin fraction is not exposed to the denaturing influence of an extensive air-water interface.

3. Complement reconstituted by addition of distilled water to the dried product is indistinguishable in appearance and hemolytic titer from the original.

4. Once the complement becomes frozen the drying process requires no supervision and thus a dried product may be obtained with only a fraction of the labor required for the method previously used.

One pound of dry calcium chloride will dry 75 c.c. of complement and leave a 50 per cent margin of safety. When employed for the Wassermann reaction, dried complement has the advantage of providing a standard product which represents the pooled serum of a number of guinea pigs, which permits of tests being carried out on many different dates with the same batch and which reduces wastage to a minimum.

BLOOD GROUPING: Rapid Method of Titrating, Pecorella, G., and Angeleri, C. Rif. Med. **47**: 8, 1931.

The iso-agglutinating titer of positive serums is tested thus: With an ordinary pipette for red corpuscles one aspirates the serum up to the mark ½; then the blood of a person of the proper group is aspirated up to the mark 1; then one aspirates up to 101 a 4.5 per cent solution of sodium citrate. The serum is thus diluted a hundredfold. The mixture is thoroughly shaken and then placed in a tube for hemolysis. This is done for the same serum with numerous persons of the same group, for the purpose of testing the potency of the serum with respect to numerous corpuscles. The tubes are put in a thermostat for an hour at 37° C. and then a microscopic examination in the hanging drop is done. Those that present evident agglutination are considered active from the point of view of their employment for the practical determination of blood groups. Using a pipette for white corpuscles and the same technic, the amount of panagglutinins is determined. The mixtures kept under refrigeration for an hour should show no trace of agglutination.

CHLOROFORM: Recovering From Brain, Lungs, and Liver, Gettler, A. O., and Blume, H. Arch. Path. **11**: 584, 1931.

Isolation of Chloroform From Tissues: The brain, lungs, and liver, as soon as removed from the body, are placed in a clean jar, sealed air-tight, and placed in the refrigerator. When the material is ice cold, about 150 gm. of the tissue to be examined is ground up. From this minced material, 100 gm. is weighed out and quickly placed in a 500 c.c. distillation flask; about 100 c.c. of water is added, also a little tartaric acid, enough to make the mixture acid in reaction. The material is then subjected to steam distillation, a long, well-cooled condenser and an adapter being used. The receptacle for collecting the distillate, a 300 c.c. Erlenmeyer flask, should contain about 10 c.c. of ice cold, acidified

(HCl) water and should be packed in ice. The tip of the adapter should reach into this ice cold water contained in the distillate receptacle. With these precautions there should be no loss of chloroform during distillation. Experiments have proved that if 250 c.c. of distillate is collected, all of the chloroform originally present in the tissue will have passed into the distillate. The distillate is well mixed, measured, stoppered, and kept cold. For the colorimetric determination 5 c.c. portions of the distillate are taken.

Preparation of Standards for Use in Colorimetric Estimation of Chloroform in Distillate: R-C-halogen$_3$ compounds, such as bromoform, chloretone, chloral and iodoform, also give the Fujiwara color reaction. It is therefore essential at the outset to determine whether chloroform or some other one of the aforementioned group is present. The authors are engaged in the standardization of a method for the determination of the foregoing series of compounds in human tissue.

The materials necessary for the colorimetric determination of chloroform in tissues are: chemically pure pyridine (colorless) 20 per cent sodium hydroxide solution and standards such as are described in the following paragraphs.

The chloroform standards are made as follows: One gram of pure chloroform is weighed out. This is best done by placing a little more than 1 gm. of chloroform in a weighing bottle having a ground glass stopper. The chloroform is allowed to evaporate spontaneously, being weighed from time to time (with weighing bottle stoppered) until the weight of chloroform in the bottle is just 1 gm. This gram of chloroform is then dissolved in about 900 c.c. of water contained in a 1 liter volumetric flask; 5 c.c. of hydrochloric acid is added and, finally, enough water is added to bring the total volume to exactly 1 liter; the whole is then thoroughly mixed. Precautions must be taken to avoid evaporation of the chloroform during the transfer. The solutions contain 1 mg. of chloroform in 1 c.c. From this stock solution a series of weaker standards are made having the following chloroform values: 0.5, 0.1, 0.01, 0.005, and 0.0025 mg., respectively, in 1 c.c. These standards, even if kept in the refrigerator, are good for only one week.

Permanent colored glass standards (an alternate standard) are prepared as follows: A series of glasses with varying depths of orange-pink color, simulating the different depths of colors obtained by various concentrations of chloroform when subjected to the pyridine test, are selected. Small disks of these glasses are cut and fitted into the circular openings of the wheel of the Hellige-Klett colorimeter, comparative type. (These colored disks are then standardized by chloroform solutions of known concentrations. The color disks 1 to 8, respectively, have the following values: Chloroform in 5 c.c. of solution, 0.125 mg., 0.25 mg., 0.375 mg., 0.5 mg., 1 mg., 1.5 mg., 2 mg., 2.5 mg. These colored glass standards keep indefinitely if kept in the dark.

Colorimetric Estimation of Chloroform in Distillate: The colorimetric method, using the chloroform standards, is applied as follows: Into five clean, dry, 50 c.c. test tubes (Folin digestion tubes may be used), 5 c.c. portions of the five standards containing respectively, 0.5 mg., 0.1 mg., 0.01 mg., 0.005 mg., and 0.0025 mg. of chloroform in 1 c.c. are measured. Into a similar test tube, 5 c.c. of the distillate is pipetted. To each of the tubes is added 5 c.c. of chemically pure (colorless) pyridine, and then 10 c.c. of 20 per cent sodium hydroxide; the contents of the tubes are mixed thoroughly; the tubes are corked loosely to avoid evaporation. Then the tubes are placed in a boiling water-bath for exactly one minute and then cooled quickly with running water. Now exactly 20 c.c. of water is added to each tube and mixed. The color of the unknown is compared with the colors of the five standards. Should the unknown match the color of the standard containing 0.01 mg. in 1 c.c. (0.05 mg. in 5 c.c.) the chloroform content of the entire distillate (250 c.c.) would be 50 times 0.05 mg., or 2.5 mg. Since 100 gm. of tissue was used in getting the distillate and the recovery is 92 per cent (see under heading "Percentage of Recovery") there are 2.5 times 1.087 or 2.72 mg. of chloroform in the 100 gm. used for analysis. If none of the five standards exactly matches the color of the unknown, the latter is compared in a colorimeter with that one of the five standards which has a color depth closest to the unknown. From the readings on the colorimeter, the chloroform content in 100 gm. of tissue is calculated as follows:

$\frac{\text{Standard}}{\text{Unknown}} \times$ Value of standard used in mg. of chloroform $\times \frac{250}{5} \times 1.087$ (correction for percentage of recovery) = mg. of chloroform in 100 gm. of tissue.

The colorimetric method, using colored glass standards, is especially adapted where routine determinations of chloroform are being done over long periods. The standard glasses are permanent. This method eliminates the preparation of a series of chloroform standards for each and every chloroform determination. It is applied as follows: Into a clean, dry test tube 5 c.c. of the distillate is pipetted; 5 c.c. of chemically pure (colorless) pyridine is added and then 10 c.c. of 20 per cent sodium hydroxide; the contents of the test tube are then mixed; the test tube is corked loosely to avoid evaporation. The tube is placed in a boiling water-bath for exactly one minute and then cooled quickly in running water. Now exactly 20 c.c. of water is added and mixed. This colored solution is placed in the cell of the Hellige-Klett colorimeter. Then the wheel of the instrument is turned, the color of each disk being compared with that of the solution in the cell. The disk that exactly matches the color of the solution in the cell is noted. The value of this disk in terms of milligrams of chloroform is known (see previous paragraph on permanent colored glass standards). Calculation:

Value of colored disk in mg. chloroform $\times \frac{250}{5} \times 1.087$ (correction for percentage of recovery) = mg. chloroform in 100 gm. tissue.

BLOOD UREA NITROGEN: Determination by Direct Nesslerization, Looney, J. M. J. Biol. Chem. **188:** 189, 1930.

Looney utilizes the protective action of a colloid (gum ghatti) in the preparation of a clear Nessler's solution.

The details of the method follow:

Preparation of the gum ghatti solution:

Twenty gm. of soluble gum ghatti are suspended on a copper gauze just below the surface of the liquid in a liter graduate filled with distilled water. After standing for twenty-four hours at room temperature the solution is filtered and is then ready for use as a stock solution.

The determination of the urea is made as follows: 5 ml. of blood filtrate, 2 drops of phosphate buffer, and 2 drops of concentrated urease solution are placed in a clean test tube in a water-bath at 55° for thirty minutes. The solution is then transferred to a urea tube graduated at 12.5 and 25.0 ml. and rinsed three times with a little distilled water so that the volume does not exceed 10.0 ml. Then 1 ml. of diluted gum ghatti solution, prepared by diluting the stock solution 1 to 8, is added and thoroughly mixed. Finally 1.5 ml. of Nessler's solution are added, the volume made up to 12.5 ml. with distilled water, and the solution again mixed. The standard is prepared by placing 3 ml. of ammonium sulphate solution containing 0.3 mg. of nitrogen in a 50.0 ml. volumetric flask and adding 8 drops of urease and 4 ml. of the diluted gum ghatti solution. The solution is then diluted to about 40.0 ml. and after it is mixed thoroughly, 6.0 ml. of Nessler's solution are added and the flask filled to the mark. It will be noted that the concentration of the solutions is twice that usually used. This was done to compensate for the decrease in the intensity of the color as the light yellow colors are rather difficult to read.

Gastric Analysis in Childhood, Dietrich, H., and Shelby, D. C. Am. J. Dis. Child. **41:** 1086, 1931.

The figures obtained for free hydrochloric acid and total acidity in the gastric contents of healthy children present wide variations. A normal figure cannot be stated.

The fractional method of gastric analysis is decidedly preferable in children. It produces less trauma, it is definitely more easily performed, and it probably gives more accurate information regarding the acidity of the gastric juice than the Ewald method.

The use of histamine marks an advance in the knowledge of gastric secretion.

Histamine stimulation should be employed in all patients who show a definitely low secretion of acid by the usual method.

The value of gastric analysis in children will be enhanced by more studies and would be greatly benefited by further improvement in present methods.

SCARLET FEVER: Results of Experimental Research, Toyoda, T., and Moriwaki, J., Futagi, Y., and Kuroi, C. Am. J. Dis. Child. 41: 1009, 1931.

The authors present the following comments:

1. A material argument against the streptococcal theory is the fact that the scarlatinal streptococcus is difficult to distinguish from other hemolytic streptococci from morphologic, biologic, and serologic standpoints. How can this objection be met?

To say that the scarlatinal streptococcus has no specificity distinguishing it from other streptococci might be construed to imply that any hemolytic streptococcus may cause scarlet fever.

As a matter of fact, it does not seem that all streptococci can cause scarlet fever. Cases were observed in which scarlet fever could be artificially produced with the streptococcus, but this point still remains to be explained more clearly. However, our investigations as to the power of hemolytic streptococci obtained from different sources to produce the toxin and to cause the scarlatinal rash by the Dick toxin have shown that not all streptococci possess this ability, and also that the degree of ability varies remarkably. Because it bears the closest resemblance to the scarlatinal streptococcus in this respect, we are induced to think that the erysipelas streptococcus, if possessed of a high degree of virulence and capable of producing the Dick toxin, can probably cause scarlet fever in a greatly susceptible person.

2. The immunity secured by infection with scarlet fever is lasting, but the same thing cannot be said about the immunity caused by other streptococcal diseases. How can this fact be explained?

As immunity from scarlet fever is an antitoxic immunity resulting from a general reaction to the scarlatinal streptococcus toxin that has spread all over the body, the antitoxin produced has the power to neutralize the streptococcus toxin; for this reason, no rash originating from the toxin can be produced. In other words, immunity to scarlet fever is permanent; however, those who have once become infected with any other streptococcal disease, such as scarlatina sine exanthemate, angina or erysipelas, are considered liable to further infection in the form of a localized disease caused by the streptococcus itself or its toxin, in spite of the presence of the antitoxin.

3. Why is there no rash in puerperal fever, phlegmon or any other streptococcal disease or in diphtheria accompanied by streptococcic infection?

We cannot state that nonscarlatinal streptococci always produce the Dick toxin. Even granting that they have such power, the degree of their ability is not necessarily the same as that ascribed to the scarlatinal streptococcus. Even the streptococcus with a high degree of ability to produce the Dick toxin must occur under suitable conditions. The principal reason that no rash appears is said to depend on the resistance of the body or the degree of its susceptibility, that is, the power of the subject's serum to neutralize the toxin. Therefore, in a highly susceptible person, even nonscarlatinal hemolytic streptococci can cause the rash, provided that they have sufficient power to produce the toxin and are kept under conditions favorable to the production of the toxin, as in puerperal scarlet fever.

4. Why is scarlet fever localized in certain fixed districts whereas streptococcal disease appears everywhere?

Scarlet fever is of comparatively minor importance in districts having a climate and other natural factors comparatively unsuited to the vitality and existence of the hemolytic streptococcus or to the production of the Dick toxin, and also in the tropics and regions inhabited chiefly by a race that enjoys a natural immunity to the streptococcus toxin.

In regions in which climatic and other conditions are favorable to the existence and vitality of the scarlet fever streptococcus and to the production of the Dick toxin and in districts inhabited by people with a high degree of susceptibility such as northern Europe, scarlet fever acquires a serious significance. Of course, streptococcal diseases appear in

other regions also, but as scarlet fever requires the conditions mentioned, the number of cases of this disease seems to vary with locality. However, once the conditions for its outbreak are fulfilled, scarlet fever can appear anywhere (e.g., the outbreak of scarlet fever in the Loochoos or Okinawa Prefecture, Japan, at the time of an epidemic of streptococcal disease). To determine the districts in Japan that may be called free from scarlet fever in a strict sense requires particularly close investigation.

5. How can the presence of scarlet fever be demonstrated in the throat and in any other focus or tissue where the presence of the streptococcus cannot be proved?

In a few cases of surgical scarlet fever, no hemolytic streptococci have been detected, the only microorganisms noted being the hemolytic staphylococci. However, in such cases the rash produced is clinically distinguishable from the scarlatinal rash. Moreover, injection of streptococcus antitoxin in the area of the rash does not cause the blanching observed in scarlatinal rash. Plainly, from the dual standpoint of the Schultz-Charlton phenomenon and the Dick reaction the rash caused by the staphylococcus toxin seems distinct from the scarlatinal rash. Accordingly, the hemolytic streptococcus can be isolated in 100 per cent of cases of ordinary scarlet fever, as demonstrated by experiments conducted not only at this hospital, but also by Dr. Kondo at the Isolation Hospital, Komagome, Tokyo, and by Dr. Nagatake at the Tokyo Imperial University Hospital. Of course, the percentage varies with the method used to obtain swab cultures. The distribution of the streptococci in the throat is shown by the researches of Dr. C. Juroi. According to his experiments, the streptococci are not detected on one side of the tonsil, whereas they occur in large numbers on the other side.

These facts show the necessity of exercising care in collecting the material from throats and in cultivating it by the pouring method. To apply the throat swab to the blood agar plate merely, as some investigators have done, will lower greatly the rate of positive results. If due attention is paid to the point mentioned and if the examinations are repeated it is believed that positive results will be obtained in 100 per cent of cases.

6. Why do other streptococcal diseases not show the phenomenon observed in scarlet fever, namely, susceptibility at its highest point in children between 3 and 8 years of age, decreasing gradually as they grow older?

As the chief initial symptoms (rash, vomiting, fever, etc.) of scarlet fever at an early stage are caused, in most cases, not by the streptococcus itself but by the absorption of its toxin, persons who possess in the blood the antitoxin capable of neutralizing the toxin (mostly adults) may contract scarlatina sine exanthemate, but will have no rash. In other words, ordinary scarlet fever accompanied by rash may occur in many infants and children and even in some adults, such as those with strongly positive Dick reactions, whose serum will produce the negative Schultz-Charlton phenomenon when injected. However, such localized changes as are observed in cases of angina, phlegmon, erysipelas, and other streptococcal diseases can be caused by the streptococcus itself or by its toxin, in spite of the presence of the antitoxin. The fact that there is not such a close relation to susceptibility to scarlet fever in these diseases as in scarlet fever itself is offered as the reason why age is of little importance in the streptococcal diseases mentioned.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Diseases of the Gall Bladder and Bile Ducts**

THIS volume is a distinct and authoritative contribution to a very important subject for, if statistics are to be relied upon, diseases of the biliary tract are exceedingly common.

The authors are and have been closely associated with the development and practical applications of cholecystography, are well equipped for the task they set out to accomplish to present in a clear and comprehensive monographic fashion the present status of diseases of the biliary tract.

The table of contents bears witness to the thoroughness with which the book has been planned and the text is the result of painstaking analysis in the light of experience.

The six chapters of Section One present discussions of the anatomy and physiology of the biliary tract; the pathology of cholecystitis; gall stones; and the symptoms and clinical diagnosis of diseases of the gall bladder and biliary ducts.

The next section discusses in detail the radiology of the biliary tract with the full technic of cholecystography and a clear presentation of its clinical interpretation and applications.

In succeeding sections the application and interpretation of tests of hepatic function are comprehensively covered, the final section describing the surgical treatment of biliary tract diseases.

The volume is an exceedingly useful and valuable contribution.

Modern Surgery†

IN THIS tenth edition of DaCosta's *Modern Surgery* the authors have themselves revised several sections. The only exception is the contribution by Chevalier Jackson, which they have allowed to remain untouched.

There are no sections on x-ray and radium as the authors deem the subjects too extensive in application for any textbook on general surgery.

This new work has been done in its usual comprehensive form and accustomed excellency.

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*Diseases of the Gall Bladder and Bile Ducts. By E. A. Graham, M.D., Professor of Surgery, Washington University, St. Louis; W. H. Cole, M.D., Instructor in Surgery, Washington University; G. H. Copher, M.D., Asst. Professor of Surgery, Washington University and S. Moore, M.D. Professor of Radiology, Washington University. Octavo, 477 pages with 224 engravings and 8 colored plates, Cloth. Lea and Febiger, Philadelphia, Pa.

†Modern Surgery. By J. Chalmers DaCosta, M.D., LL.D., F.A.C.S., Samuel D. Gross, Professor of Surgery, Jefferson Medical College, Surgeon to Jefferson Medical College Hospital, Consulting Surgeon to the Philadelphia General Hospital, St. Joseph's Hospital and Misericordia Hospital, Philadelphia. Assisted by Benjamin Lipshutz, M.D., F.A.C.S., Surgeon to the Mt. Sinai Hospital; Associate in Neuro-anatomy, Jefferson Medical College. Tenth Edition, Revised and Reset. 1,404 pages with 1,050 illustrations, some in colors. Cloth, W. B. Saunders Company, Philadelphia and London, 1931.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*Annals of the Pickett-Thomson Research Laboratory**

EVERYONE is familiar with the Monographs issued periodically from the Pickett-Thomson Research Laboratory the purpose of which, eventually, is to record photographically the whole range of bacteria and protozoa.

Volume VI (Monograph XI) is devoted entirely to the rôle of the streptococci in scarlet fever and embodies, not only a comprehensive analytical review and summary of the literature derived from about 1400 research papers, but also the independent investigations of these laboratories.

The amount of material thus correlated is stupendous and the volume presents practically all the evidence available concerning the subject.

It is, in effect, also a technical manual for the study of streptococci in scarlet fever and as such alone will be invaluable as a reference text in the bacteriologic laboratory.

It would be impossible within reasonable limits to summarize, or, indeed, even to tabulate the material assembled and discussed. Suffice it to say that if there are any significant omissions in the work done in this field they were not apparent to this reviewer.

The monograph is comprehensive in that it summarizes, not only the bacteriologic studies which have been made in scarlet fever but also related investigations such as hematologic and immunologic studies, reports of the significance of blood sedimentation, precipitin, and complement-fixation tests and so on.

As a result of the evidence so painstakingly assembled, so carefully studied, analyzed, and to a large extent independently corroborated, the authors are convinced that scarlet fever is caused by a definite, specific variety of hemolytic streptococci, Streptococcus scarlatinae.

This conclusion is based upon evidence thus briefly summarized:

1. Cultural differences, explained in detail as well as illustrated, together with the methods whereby they may be demonstrated.
2. Fermentation and other chemical differences.
3. Agglutination differences.
4 and 5. Differences shown by the opsonic index and precipitin tests.
6. Specificity of the toxin.
7. Production of rash by the toxin.
8. Production of rash by vaccine.
9. Schultz-Charlton phenomenon.
10. Curative effect of antiserum.
11. Passive immunization by antiserum.
12. Immunization by toxin and vaccine.
13. Experimental production of scarlet fever.
14. Presence of S. scarlatinae in udders where epidemics have been due to milk.
15. Laboratory infections.

Abundant evidence is presented under all these headings.

An extensive bibliography is appended.

The illustrations, all microphotographs, are excellent and excellently reproduced.

There is only one criticism, which this reviewer has made before: It seems a pity that such useful monographs should be bound in paper covers. They deserve a more permanent preservation.

A Textbook of Pathology†

THIS is an eminently practical text in which the essential facts of pathology are presented in such a fashion as to correlate the structural changes with the clinical features of disease.

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*Annals of the Pickett-Thomson Research Laboratory. Volume VI. The Pathogenic Streptococci: The Rôle of The Streptococci in Scarlet Fever. By D. Thomson, Director, and R. Thomson, Pathologist, Pickett-Thomson Research Laboratory. Paper, 470 pages, 13 plates. Williams and Wilkins Co., Baltimore, Md.

†A Textbook of Pathology. Edited by E. T. Bell, M.D., Professor of Pathology, University of Minnesota, Contributors: E. T. Bell, M.D., Professor of Pathology, B. J. Clawson, M.D., Professor of Pathology, Hal Downey, M.D., Professor of Hematology, J. S. McCartney, M.D. Asst. Professor of Pathology, J. C. McKinley, M.D. Prof. Neuropathology and Neurology, C. J. Watson, M.D., Instructor in Pathology, University of Minnesota, Minneapolis, Minn. Cloth, 627 pages, 316 engravings, and 2 colored plates. Lea and Febiger, Philadelphia, Pa.

While intended primarily for the medical student it may be read and consulted with profit by the practitioner at large who, while he is confronted with the results of disease, must nevertheless comprehend the underlying mechanism which produces the symptoms.

The book is generously and excellently illustrated and can be looked upon as an excellent and practical reference text.

Its format is in keeping with the usual pleasing standard of these publishers.

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*Textbook for Nurses**

THE original purpose of this book was to provide the nurse with a comprehensive textbook in which broad principles were outlined without undue emphasis on technical terms.

The fact that it has gone through thirteen printings and four editions is prima facie evidence of its utility as a reference text.

Section I covers Anatomy and Physiology in sixteen chapters; Section II devotes eighteen chapters to Surgery; Medicine is covered in twelve chapters; Chapter XIV of this section is given to Hygiene, Chapter XV to Clinical Instruments and Methods; and Chapter XVI to Poisons and Antidotes.

A Table of Weights and Measures is included.

The book is well indexed and can be recommended as a comprehensive reference text.

Histology†

THE authors, in the Preface, state that "we have attempted to describe the tissues of the body in such a way that the student will be given an adequate knowledge without being burdened with details which only a specialist would require. We have endeavored to be lucid and at the same time to avoid lengthy descriptions."

In accordance with this plan the subject is presented by concise descriptions and clear diagrams, the majority of which are camera lucida drawings in color.

The result is a unique textbook of histology of inestimable value to all whose interests lie in this field. The work represents an authoritative text presented in a manner most creditable to the bookmaker's art.

Both the drawings and the text are clear and represent the actual experience of the authors.

The book is a distinct and valuable contribution to the subject and will appeal, not only to those who approach the subject de novo, but also to those who desire to check their recollection or refresh their memory.

There is an excellent and very full index.

Bacteriological Technique‡

IT IS doubtful if there is any worker in bacteriology who, since the first edition appeared in 1902, has not found Eyre's manual an exceedingly useful book to have handy.

This latest tenth edition will be received, therefore, as an old and tried friend.

It has been amply revised and extended to incorporate the newer methods which have withstood, or give promise of withstanding the test of time and trial.

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*Textbook for Nurses, Anatomy, Physiology, Surgery and Medicine. By E. W. H. Graves, Professor of Surgery, University of Bristol and J. M. Fortescue-Brickdale, Clinical Lecturer, University of Bristol, Medical Section Revised by J. A. Nixon, Professor of Medicine, University of Bristol. Ed. 4, Cloth, 641 pages, 229 figures. Oxford University Press.

†Histology, for Medical Students. By H. Hartridge, Professor of Physiology, University of London, and F. Haynes, Demonstrator of Histology, University of London. Cloth, 400 pages, 259 colored plates containing 512 figures. Oxford University Press, New York.

‡Bacteriological Technique. By J. W. H. Eyre, Professor of Bacteriology, University of London. Cloth, 619 pages, 237 figures. William Wood and Co., New York.

The majority of the references, as, perhaps, is natural, are to the work of European investigators which, however, sometimes lead to omissions. Under the Gram stain, for example, there is no reference to the work which has been done concerning the influence of reaction upon the results obtained with the Gram technique nor to the various buffered solutions evolved to overcome these difficulties.

Under the preparation of the Jenner stain, also, the necessity for thorough, and at times even prolonged, washing of the precipitate, is not emphasized.

These, however, are but minor defects. The book remains, as before, a reliable, authoritative, and standard text.

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*Chemical Methods in Clinical Medicine**

THIS book is addressed principally to the student and practitioner and has for its purpose the description of the technic of simpler laboratory methods which do not require extensive laboratory equipment, as well as some discussion of the clinical significance of their results.

The first sixteen chapters are concerned with the examination of the urine and of urinary deposits, and a description of the simpler tests of renal function.

As natural offshoots, so to speak, of the subject this portion of the book also includes, following the consideration of reducing substances in the urine, a discussion of the interpretation of blood-sugar curves, chemical tests in diabetes and the control of insulin treatment, and ketosis, acidosis, and alkalosis.

The remainder of the book covers chemical examinations of the blood, chemical examination of the spinal fluid, milk analysis, gastric contents, chemical examination of duodenal contents, chemical examination of the feces, basal metabolism, and a chapter on miscellanea.

An appendix contains an alphabetical list of reagents and the methods for their preparation, together with useful tables and suggestions concerning the care of laboratory glassware, etc. There is a complete index.

Throughout the book the calculations involved are presented in full detail because, as the author says in his preface, more mistakes are made in calculations than in technic.

Heading each chapter is a list of references from which additional information may be sought.

The practitioner, and especially the practitioner without ready access to laboratory facilities, should find this a very useful book.

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*Chemical Methods in Clinical Medicine. By G. A. Harrison, Reader in Chemical Pathology, University of London. Cloth, 534 pages, 64 illustrations, one colored plate. The Macmillan Co., New York.

ALDRED SCOTT WARTHIN, M.D., PH.D., LL.D.
1866-1931

The Journal of Laboratory and Clinical Medicine

VOL. XVI ST. LOUIS, MO., JULY, 1931 No. 10

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

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Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIAL

ALDRED SCOTT WARTHIN (1866-1931)

AN APPRECIATION

AT FOUR o'clock on Saturday morning, May 23, 1931, death came unexpectedly at his home in Ann Arbor to one of the most illustrious of the medical scientists of our generation. Two months earlier he was taken ill while attending, in Baltimore, the annual meeting of the American College of Physicians and he left the hospital and returned to his home only a week before his death. To but few is it given to leave upon the science of medicine so strong and enduring an impress as that of Doctor Warthin.

He was a great teacher; from the first day of class work he inspired and profoundly impressed those who sat before him, that no shallow, superficial thinking would be tolerated. His was a critical attitude of mind with a deep sincerity of purpose. His dealing with each subject was thorough, complete and exact. From his wealth of experience and broad interests he brought into

teaching pertinent remarks from the fields of music, art, religion, natural science, and history but, as is so frequently the case, these digressions had a greater significance in the moulding of the life of the student than the subject they were intended to embellish. The philosophical presentation of his subject attracted many visitors from the non-medical group to his classroom and the championship of clean living left its impress.

Dr. Warthin was a man of boundless energy and industry. It was only natural for him to exact the same high standards of achievement in others. His students at times were resentful of the demands to which they were subjected, but they were appreciative of the thoroughness and value of the instruction. No small number of those who thought the burden unbearable during the ordeal, later returned with unstinted gratitude for the firm foundation they had established in working objectively, and in accuracy of observation under his tutorage.

Born in Greensburg, Indiana, in 1866, Doctor Warthin received a Teacher's Diploma from the Cincinnati Conservatory of Music in 1887; his A.B. degree from Indiana University in 1888, where he came in contact with David Starr Jordan; from the University of Michigan, A.M. in 1890, M.D. in 1891, Ph.D. in 1893. He was honored by the Indiana University in 1928 with the LL.D. degree. After taking his medical degree Dr. Warthin became associated with Professor Dock as Demonstrator of Clinical Medicine in which position he served for four years. It was during this period that he acquired a firm foundation in diagnosis and observation. In 1895 he was transferred to the department of Pathology where his unusual ability found free scope for development. He became Instructor in Pathology in 1897, Assistant Professor in 1899 and Junior Professor in 1902. Finally in 1903 he was made Professor of Pathology and Director of the Pathological Laboratory which position he held up to the time of his death.

But he was not only a teacher, he was a patient and careful investigator and his published papers reveal him at his best. His writings are marked by clarity, accuracy of observation, an easy flow of language, sound reasoning, and an all pervading sanity.

As might be expected his earlier papers deal with his experiences in Internal Medicine in which we find a generous distribution between bedside and laboratory studies. The limited number of autopsies available in the nineties led to an intensive study of the, hitherto, relatively unknown fields. In 1901 there appeared the first of a series of nine papers on the histology and pathology of the hemolymph nodes. The originality and completeness of these studies were such that the name of their author will always be associated with our conception and function of the hemolymph and splenolymph nodes. In 1904, and again in 1906, he developed and defended a theory of the interrelationship of the lymphoblastomatous conditions which is only at the present time receiving general acceptance. The studies of Dr. Warthin upon the blood-forming organs proved him to be the forerunner of the present-day conceptions of the reticulo-endothelial apparatus. His early recognition of the importance of constitutional pathologic types in the etiology of disease, which he stressed in his teaching, anticipated the more recent studies on the

etiology of neoplasia, of pernicious anemia, and of so-called hyperthyroidism. The year 1908 marked the beginning of a period of active and productive interest in tuberculosis.

In 1910 the first of the studies on the pathology of syphilis appeared. Dealing with congenital syphilis at first, the essential unity of the pathology of this disease in various stages and in various organs was soon recognized and a large series of contributions, extending to the present time, has established upon a sound basis, a new conception of the pathology of latent syphilis; especially as this condition is to be found in the heart, aorta, pancreas, adrenals, and testes. His development of rapid staining methods for the demonstration of the causal organisms in active foci in the clinically latent cases forced conclusions which, at first, were unwelcome to therapists who based their findings on the gross pathology. He was persistent, and once having reached a conclusion he had no hesitancy in expressing his views—to this, much of his greatness can be ascribed.

It is said of Buddha that after he had learned all there was to be learned he went into retreat and considered whether to give the world his knowledge or keep it to himself. The active participation of Dr. Warthin in the scientific programs of the many societies in which he held membership, his frequent appearance before medical groups gave him opportunities to discuss and defend the results of his investigations. On such occasions he was prone to be positive and dogmatic but he welcomed criticism and a partially concealed smile and twinkle of the eye would reveal his love of controversy. Woe unto the unfortunate one who found himself poorly founded in the history and pertinent literature of his subject. Dr. Warthin readily assumed his share of the burden of administration of the scientific societies to which he belonged as the following list of offices held in these organizations will show.

American Association of Pathology and Bacteriology, President in 1908; Michigan Association for Prevention and Relief of Tuberculosis, Secretary in 1907-1918 and President, 1918-1919; Ann Arbor Anti-Tuberculosis Association, Secretary and President, 1917-1918; Michigan Social Hygiene Association, President, 1917-1918; International Association of Medical Museums, President, 1910-1913, Vice-President, American Section, 1914, Member of the Council for the United States, 1914-1927, Editor, Bulletin, 1913-1919; Association of American Physicians, President, 1928; American Association of Cancer Research, President, 1928; Association of Experimental Pathology, President, 1924; Delegate to National Research Council, 1925-1928; American College of Physicians, Master, and First Vice-President since 1925; Editor, Annals of Clinical Medicine since 1924; American Medical Association; International Society of Medical History, President, 1930-1931; Chicago Medical History Society; Corresponding Member of International Association for the Study of the Prevention of Tuberculosis.

As a pioneer in the organization and administration of a diagnostic and teaching department his routine methods became models for others. As might be expected, his advice and opinions were eagerly sought in cases of questionable diagnosis, and he gave unstintingly of his time as an expert witness in the furtherance of justice, the major portion of which went unrewarded.

He early incorporated a museum in the planning of his department and it now stands as a monument to his genius.

His devotion to science was augmented by an appreciation of art and literature. A few weeks before his death he had the great satisfaction of receiving his last publication, *The Physician of the Dance of Death*. In this volume he has brought together, sparing neither time nor expense, the art of the Middle Ages, a subject which intrigued and fascinated him from his student days to the very end.

No appreciation of Dr. Warthin would be complete without reference to his philosophy of life. In his every act he was a model of clean living. More than a year ago he wrote in his *The Creed of a Biologist*, "Now as I approach the end of my teaching, I am doing what a number of these students has long importuned me to do; in this little book I am expressing my biologic credo, my philosophy of life which has animated and directed my existence, which has given meaning to the universe for me and brought me peace of mind and happiness." And thus death came as he would have planned, following a day, some hours of which were spent in his garden, where behind a massive stone wall constructed with his own hands he had for thirty years found relaxation from the confining work of the laboratory, among his beautiful flowers.

Whilst we lay this wreath of tribute at his feet, we are impressed with the conscious knowledge that among all the glowing tributes which came, he remained a true scientist, a loyal and sincere friend, a true husband and father, and he has contributed much to the health, happiness and welfare of our life.

—M. H. Soule.